8EHQ-0102-15037

American Chemistry Council *Good Chemistry Makes It Possible*

December 11, 2001

Ms. Christine Todd Whitman
Administrator
U. S. Environmental Protection Agency
P. O. Box 1473
Merrifield, VA 22116

Contain No CBI

Dear Ms. Whitman:

The American Chemistry Council (Council) makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, the following four final reports that the Council's Brominated Flame Retardant Industry Panel (BFRIP) recently conducted are enclosed:

- Hexabromocyclododecane (HBCD):
  - An Early Life-Stage Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*);
- Decabromodiphenyl Oxide (DBDPO):
  - Potential for Biotransformation of Radiolabelled Decabromodiphenyl Oxide (DBDPO) in Anaerobic Sediment;
  - A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants; and,
  - An Activated Sludge, Respiration Inhibition Test.

These reports do not include confidential information.

If you have any questions, please contact Wendy K. Sherman, the BFRIP Manager, at 703/741-5639 or via email [wendy.sherman@americanchemistry.com].

Sincerely yours,

Elizabeth Festa Watson

Elizabeth Festa Watson
Managing Director, CHEMSTAR

Enclosures (4)

MR-5H113

HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-112

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines
OPPTS Number 850.1400
and
OECD Guideline 210

AUTHORS:

Kurt R. Drottar
Jon A. MacGregor
Henry O. Krueger, Ph.D.

STUDY INITIATION DATE: August 8, 2000

STUDY COMPLETION DATE: July 12, 2001

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

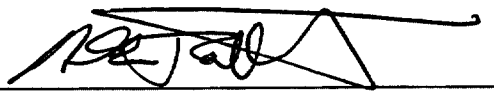
TITLE: Hexabromocyclododecane (HBCD): An Early Life-Stage Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-112

STUDY COMPLETION: July 12, 2001

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

STUDY DIRECTOR:



Kurt R. Drottar
Senior Biologist

7/12/01
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Test Substance Preparation	August 21, 2000	August 22, 2000	August 24, 2000
Test Initiation	August 24, 2000	August 25, 2000	August 29, 2000
Matrix Fortifications	September 28, 2000	September 28, 2000	September 29, 2000
Fish Lengths	November 20, 2000	November 20, 2000	November 27, 2000
Analytical Data and Draft Report	February 2 and 6, 2001	February 6, 2001	February 7, 2001
Biological Data and Draft Report	February 1 and 5 – 8, 2001	February 8, 2001	February 14, 2001
Final Report	July 12, 2001	July 12, 2001	July 12, 2001

Kimberly A. Hoxter

Kimberly A. Hoxter
Quality Assurance Representative

7-12-01
DATE

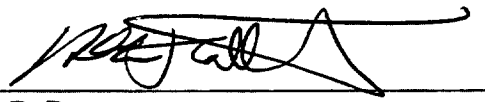
REPORT APPROVAL

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Hexabromocyclododecane (HBCD): An Early Life-Stage Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-112

STUDY DIRECTOR:



7/12/01

Kurt R. Drottar
Senior Biologist

DATE

MANAGEMENT:

7/12/01

Henry O. Krueger, Ph.D.
Director, Aquatic Toxicology
and Non-Target Plants

DATE

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SUMMARY

SPONSOR:	American Chemistry Council's Brominated Flame Retardant Industry Panel
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	439A-112
TEST SUBSTANCE:	Hexabromocyclododecane (HBCD)
STUDY:	Hexabromocyclododecane (HBCD): An Early Life-Stage Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)
NOMINAL TEST CONCENTRATIONS:	Negative Control, Solvent Control, 0.43, 0.85, 1.7, 3.4 and 6.8 µg HBCD/L
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control, Solvent Control, 0.25, 0.47, 0.83, 1.8 and 3.7 µg HBCD/L
TEST DATES:	Experimental Start – August 24, 2000 Biological Termination – November 22, 2000 Experimental Termination – November 22, 2000
LENGTH OF TEST:	88 Days

TEST ORGANISM:	Rainbow Trout (*Oncorhynchus mykiss*)
SOURCE OF TEST ORGANISMS:	Mt. Lassen Trout Farm 28125 Hwy 36E Red Bluff, CA 96080
AGE OF TEST ORGANISMS:	Newly-fertilized embryos < 24 hours old at test initiation

NO-OBSERVED-EFFECT-CONCENTRATION:	3.7 µg HBCD/L Measured (6.8 µg HBCD/L Nominal)
LOWEST-OBSERVED-EFFECT - CONCENTRATION:	>3.7 µg HBCD/L Measured (>6.8 µg HBCD/L Nominal)
MAXIMUM-ACCEPTABLE-TOXICANT-CONCENTRATION:	>3.7 µg HBCD/L Measured (Not Calculable) >6.8 µg HBCD/L Nominal (Not Calculable)

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for the American Chemistry Council's Brominated Flame Retardant Industry Panel at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The in-life phase of the test was conducted from August 24, 2000 to November 20, 2000. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 439A-112 in archives located on the Wildlife International, Ltd. site. The solubility of HBCD in water at 25°C is 3.4 µg HBCD/L. The 96-hour LC50 for rainbow trout is >6.8 µg HBCD/L based on nominal concentrations (>2.5 µg HBCD/L based on measured concentrations).

OBJECTIVE

The objective of this study was to evaluate the toxicity of hexabromocyclododecane (HBCD) during early life-stage development of rainbow trout (*Oncorhynchus mykiss*). Hatching success, time to hatch, time for larvae to swim-up, and post-hatch growth and survival were evaluated during the 88-day test.

EXPERIMENTAL DESIGN

Rainbow trout embryos were exposed to a geometric series of five test concentrations, a negative (dilution water) control and a solvent control under flow-through conditions. Four replicate test chambers were maintained in each treatment and control group, with each test chamber containing two incubation cups. The test was initiated with the distribution of newly-fertilized eggs to the incubation cups. Each incubation cup contained a nominal count of 15 embryos, resulting in a nominal total of 30 embryos per replicate and 120 embryos per experimental group. An additional 30 embryos were held in each of four incubation cups in dilution water and were sacrificed on Day 11 to evaluate the fertilization success. The total exposure period was 88 days, which included a 27-day hatching period and a 61-day post-hatch period.

Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the water solubility of the test substance. Nominal test concentrations selected were 0.43, 0.85, 1.7, 3.4 and 6.8 µg HBCD/L. A negative control and a solvent control (acetone) were also conducted concurrently. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at test initiation, at weekly intervals during the test,

and at test termination.

Delivery of the test water to the test chambers was initiated approximately 47 hours prior to the addition of the embryos to the incubation cups in order to achieve equilibrium of the test substance. To initiate the test, newly-fertilized embryos were indiscriminately distributed among incubation cups in groups of one or two until each cup contained a nominal count of 15 embryos. Two cups then were indiscriminately placed in each treatment and control test chamber. Dead embryos were removed daily until hatching began. After hatching, the larvae from all test concentrations were counted and released into the appropriate test chambers where the exposure continued for 61 days. When more than 90% of the negative control group reached the swim-up stage, the number of larvae in all replicates was reduced to 15 to prevent overcrowding.

Embryo survival (hatching success), time to hatch, time to swim-up of the larvae, and the post-hatch growth and survival were measured for the rainbow trout in each treatment and control group. Observations were made during the embryo incubation and post-hatch periods to assess the effects of the test substance on these parameters. Fish lengths were measured at 29 days post-hatch and at test termination. The wet weight and dry weight of each surviving fish were measured at test termination. Data were evaluated to determine the no-observed-effect-concentration (NOEC) and the lowest-observed-effect-concentration (LOEC). The NOEC and LOEC were used to estimate the maximum acceptable toxicant concentration (MATC).

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Hexabromocyclododecane (HBCD): An Early Life-Stage Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)"(Appendix 1). The protocol was based on procedures outlined in the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.1400 (1); OECD Guideline for Testing of Chemicals 210: *Fish Early Life-Stage Toxicity Test* (2); *Standard Evaluation Procedure, Fish Early Life-Stage Test* (3); and ASTM *Standard E1241-88a Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish* (4).

concentrations. Stock solutions were prepared 11 times during the test period. The concentration of acetone in the solvent control and all HBCD treatment groups was 0.10 mL/L. At test initiation and termination, the mixing chambers and test solutions appeared clear and colorless.

Test Organism

Newly-fertilized rainbow trout, *Oncorhynchus mykiss*, embryos were used in this test. The rainbow trout is representative of an important group of aquatic vertebrates and was selected for use in the test based upon past history of use and ease of handling in the laboratory. Unfertilized eggs and sperm were obtained from Mt. Lassen Trout Farm, Red Bluff, California. Gametes from three females and three males were used in the test. The eggs were fertilized at Wildlife International, Ltd. on August 24, 2000 and the test was initiated within four hours of fertilization.

Larval fish were fed salmon-starter mash supplied by Zeigler Brothers, Inc., Gardners, Pennsylvania, beginning on Day 49 (the end of the swim-up stage). Food was provided three times daily during the first seven days. Thereafter, larvae were fed three times per day on weekdays and at least two times daily on weekends and holidays. The fish were not fed approximately 55 hours prior to the termination of the test to allow for clearance of the digestive tracts before weights were measured. To ensure that the feeding rate per fish remained constant, rations were adjusted each week to account for losses due to mortality. Excess feed was siphoned from the bottoms of the test chambers, as needed. Biomass loading (the total wet weight of the fish per liter of test water) at the end of the test was measured in one negative control replicate and was calculated to be 0.36 g fish/L/day of test water that passed through the test chamber during a 24-hour period. Instantaneous loading was 2.3 g fish/L of test water in the test chamber at any given time.

Test Apparatus

A continuous-flow diluter was used to provide each concentration of the test substance, a solvent control and a negative (dilution water) control. Syringe pumps (Harvard Apparatus) were used to deliver the five test substance stock solutions and acetone for the solvent control into mixing chambers assigned to each treatment and control group. The stock solutions were mixed with dilution water in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters. The flow of test water from each mixing chamber was split and allowed to flow into four replicate test chambers. The proportion of test water

that was split into each replicate was checked prior to the test and at approximately weekly intervals thereafter to ensure that flow rates varied by no more than ±10% of the mean for the four replicates.

The diluter was adjusted so that each test chamber received 6.4 volume additions of test water every 24 hours. The stock solution delivery pumps were calibrated before the test, while the dilution water rotameters were calibrated before the test and at approximately weekly intervals during the test. The general operation of the diluter was checked visually at least two times per day during the test and once at the end of the test.

Test chambers were 9-L glass aquaria filled with approximately 7 L of test water. The depth of the test water in a representative chamber was approximately 18 cm. Test chambers were impartially positioned in a temperature-controlled environmental chamber. The test chambers were labeled with the project number, test concentration and replicate.

The embryo incubation cups were suspended in the water column of each test chamber and attached to a rocker arm. The reciprocating motion of the rocker arm (approximately 2 rpm) facilitated circulation of test water around the embryos during incubation. The incubation cups were constructed from glass cylinders approximately 50 mm in diameter with 425 µm nylon screen mesh attached to the bottom with silicone sealant.

Dilution Water

The water used for holding and testing was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity and pH measurements of the well water during the four-week period immediately preceding the test are presented in Appendix 3.

The well water was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank and aerated with spray nozzles. The dilution water again was filtered (0.45 µm) to remove microorganisms and particles. Prior to use, the water was passed through a UV sterilizer as an additional method of water treatment. The results of periodic analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International, Ltd. are presented in Appendix 4.

Environmental Conditions

The rainbow trout embryos/larvae were kept in darkness except during observations until one week after hatching. After this period of time, lighting used to illuminate the test chambers was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. The light intensity measured on the day the photoperiod started was 287 lux at the surface of the water.

Temperature was measured in each test chamber at the beginning and end of the test and at weekly intervals during the test (with the exception of Day 28) using a liquid-in-glass thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the study was 12 ± 1°C. Measurements of pH were made on water samples collected from alternating replicates of each treatment and control group at the beginning and end of the test and at weekly intervals during the test. Dissolved oxygen concentrations were measured daily in alternating replicates of each treatment and control group during the first seven days of the test, at weekly intervals during the test, and at test termination. Hardness, alkalinity and specific conductance were measured in alternating replicates in the negative control and one treatment level (6.8 µg HBCD/L, nominal concentration) at the beginning of the test, once a week during the test, and at test termination.

Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Specific conductance was measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter. Hardness and alkalinity measurements were made by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (5).

Biological Observations and Measurements

Daily observations were made during the embryo incubation and post-hatch exposure periods to evaluate the numbers of individuals exhibiting clinical signs of toxicity or abnormal behavior. Hatching success, time to hatch, time to swim-up of the larvae, and post-hatch survival were evaluated from these observations. Hatching success was calculated as the percentage of eggs that

hatched successfully. Post-hatch percent survival was calculated for the intervals prior to and after thinning. Post-hatch survival prior to thinning was calculated as the number of larvae alive at thinning on Day 22 post-hatch divided by the total number of larvae that had successfully hatched. Survival at the end of the test was calculated as the number of juvenile fish alive on Day 61 post-hatch divided by the number of larvae remaining after thinning.

Post-hatch growth of the rainbow trout was measured on Day 29 post-hatch and at the conclusion of the test. Fish total lengths were measured at 29 days post-hatch by the photometric method of Martin (6) using the SIGMA SCAN™ scientific measurement system. At test termination, total lengths for each surviving fish were made using a metric ruler, while wet and dry weights were measured using an analytical balance.

Statistical Analyses

Test endpoints that were analyzed statistically included: hatching success, time to swim-up, percent survival, total fish length on Day 29, and total length, wet and dry weight of the juvenile fish at test termination. Data from the negative and solvent control groups were compared using either 2 X 2 contingency tables or Student's t-test. When no differences were detected between the two control groups ($p > 0.05$), those data were pooled and used to assess treatment level effects. Hatching success, time to swim-up and percent survival were analyzed using 2 X 2 contingency tables and the chi-square test to identify treatment groups that showed a statistically significant difference ($p \leq 0.05$) from the pooled control group. Length and weight data were evaluated for normality using the Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test (7). For data which passed both homogeneity of variance and normality tests, the Bonferroni t-test (7) was used to evaluate differences between treatment and pooled control means.

The results of the statistical analyses were used to aid in the determination of the NOEC and the LOEC. All statistical tests were performed on a personal computer using TOXSTAT Version 3.5 (7) or SPSS/PC Version 2.0 (8) statistical software.

Sampling for Analytical Chemistry

Prior to test initiation, samples of water were collected from one replicate test chamber of each control and treatment group to evaluate diluter performance. During the definitive test, water samples

were collected from one alternating replicate of each control and treatment group at test initiation, at weekly intervals during the test and at test termination. All samples (50 mL) were collected from mid-depth of the chambers, placed in 125-mL separatory funnels and were analyzed immediately. Analytical procedures used in the analysis of the samples are provided in Appendix 5.

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Nominal test concentrations were 0.43, 0.85, 1.7, 3.4 and 6.8 µg HBCD/L. Prior to test initiation, water samples were collected and analyzed from one replicate test chamber of each treatment and control group to evaluate diluter performance. Concentrations of HBCD in the pre-test samples ranged from 52 to 90% of the nominal concentrations and the percent recovery tended to decrease with increasing concentration (Appendix 5, Table 3). This trend indicates that the 3.4 and 6.8 µg HBCD/L treatment groups were at or above the limit of solubility for HBCD under the conditions of administration. Pre-test concentration measurements were not used in the calculation of the mean measured concentrations achieved during the test.

Results of analyses to measure concentrations of HBCD in water samples collected during the test are presented in Table 1 and in the analytical chemistry report (Appendix 5, Table 6). When measured concentrations of samples analyzed during the test were averaged, the mean measured concentrations for the study were 0.25, 0.47, 0.83, 1.8 and 3.7 µg HBCD/L, which represented 58, 55, 49, 53 and 54% of the nominal concentrations, respectively. Mean measured concentrations were used to express the NOEC.

Physical and Chemical Measurements of Water

Measurements of temperature, dissolved oxygen and pH are presented in Tables 2, 3 and 4, respectively. All temperature data collected was within the desired range of 12 ± 1°C. Dissolved oxygen concentrations remained ≥ 6.6 mg/L (61% of saturation) and measurements of pH ranged from 7.6 to 8.1. Measurements of conductivity, hardness and alkalinity in the negative control and the 3.7 µg HBCD/L treatment group are presented in Table 5. No apparent differences in these parameters existed between the negative control and the treatment group.

Percent Fertilization

Egg viability was determined on Day 11 from embryos maintained in dilution water under test conditions. Water quality measurements in the fertilization control (collected between Days 0 and 7) were comparable to the actual test chambers. Temperature ranged from 11.3 to 12.1°C. Dissolved oxygen concentrations remained ≥8.4 mg/L (78% of saturation) and measurements of pH ranged from 7.9 to 8.1. To determine egg viability, embryos were removed from the fertilization control test chambers and placed in 10% glacial acetic acid. Embryos were considered viable (fertilized) if the presence of a neural keel was observed. Percent fertilization was calculated by dividing the number of embryos with a neural keel by the total number of eggs. Mean percent fertilization was 99% (Table 6).

Time to Hatch and Hatching Success

Daily observations of embryos and newly hatched larvae indicated that there were no apparent differences in time to hatch between the control groups and any of the HBCD treatment groups (Table 7). Rainbow trout embryos began hatching on Day 23 and all surviving embryos in the control and treatment groups had hatched by Day 33. Based upon the number of dead embryos removed and the number of live larvae, it was concluded that two replicates had received the incorrect number of embryos at test initiation: 1) 32 embryos were exposed in replicate B of the negative control, and 2) 31 embryos were exposed in replicate C of the 3.7 µg HBCD/L treatment group. In these cases, hatching success was calculated using totals of 122 and 121 exposed embryos, respectively. Hatching success in the negative control and solvent control groups averaged 75 and 85%, respectively. A 2 X 2 contingency table showed that there was no statistically significant ($p > 0.05$) difference between the negative and solvent control and the controls were pooled for comparisons among the treatment groups. Hatching success in all HBCD treatment groups was ≥83% and was not significantly different from the pooled controls ($p > 0.05$). Consequently, the NOEC for hatching success was 3.7 µg HBCD/L, the highest concentration tested.

Time to Swim-Up

The swim-up stage is the period of time when the fish begin to actively swim. Time to swim-up was determined from daily observations of the fish. Rainbow trout larvae began swimming up from the bottom of the test chambers on Day 13 post-hatch. By Day 22 post-hatch, 97% of the negative control fish had attained swim-up (Table 8). At this time, all test chambers were thinned to

15 fish. A 2 X 2 contingency table showed that there was no statistically significant ($p > 0.05$) difference between time to swim-up in the negative and solvent control and the controls were pooled for comparisons among the treatment groups. There were no statistically significant reductions in the numbers of fish swimming up in any HBCD treatment group in comparison to the pooled controls ($p > 0.05$). Consequently, the NOEC for time to swim-up was 3.7 µg HBCD/L, the highest concentration tested.

Larvae and Fry Survival

Rainbow trout survival was analyzed for two time periods: 1) Day 1 post-hatch to thinning on Day 22 post-hatch (Table 9) and 2) Day 22 post-hatch to Day 61 Post-hatch (Table 10). In both time periods, survival in the negative and solvent control groups were not significantly ($p > 0.05$) different and the controls were pooled for comparisons among the treatment groups. Mean control survival prior to thinning was 97%. Mean survival prior to thinning in the HBCD treatment groups was ≥97% and was not significantly different in comparison to the pooled controls ($p > 0.05$). Mean control survival after thinning was 98%. One fish in the D replicate of the solvent control was inadvertently killed during siphoning on Day 66 of the test (Day 39 post-hatch). In addition, one fish jumped out of the holding bucket during cleaning of the test chambers on Day 82 of the test (Day 55 post-hatch). These fish were excluded from the calculation of survivorship for the replicates. Mean survival after thinning in the HBCD treatment groups was ≥97% and was not significantly different from the pooled controls ($p > 0.05$). Consequently, the NOEC for larvae and fry survival was 3.7 µg HBCD/L, the highest concentration tested.

Biological Observations

All organisms were observed daily to evaluate the numbers of mortalities and the numbers of individuals showing sublethal signs of toxicity. All surviving fish in the negative and solvent control appeared normal and healthy during the test (Table 11). All surviving fish in the HBCD treatment groups also appeared normal and healthy during the test.

Growth

Growth data were evaluated on Day 29 post-hatch and at the end of the test (Tables 12 and 13). On Day 29 post-hatch, growth was evaluated by taking photographic slides of the fish and determining their total lengths from the slides (Appendix 6). At test termination, growth

measurements were made by direct measurement of total length, wet weight and dry weight (Appendices 7, 8 and 9, respectively). For all measurements, growth in the negative control and solvent control were not significantly different ($p > 0.05$) and the data was pooled for comparisons among the HBCD treatment groups. At Days 29 and 61 post-hatch, total length was not significantly reduced in any HBCD treatment group ($p > 0.05$). In addition, at Day 61 post-hatch, wet weight and dry weight were not significantly reduced in any HBCD treatment group ($p > 0.05$). Consequently, the NOEC for growth was 3.7 µg HBCD/L, the highest concentration tested.

CONCLUSIONS

Rainbow trout (*Oncorhynchus mykiss*) exposed to hexabromocyclododecane (HBCD) at concentrations up to 3.7 µg HBCD/L for 61-Days post-hatch showed no effects on hatching success, time to swim-up, larval survival, fry survival or growth. The reported solubility of HBCD is 3.4 µg HBCD/L. Consequently, HBCD was not chronically toxic to rainbow trout at concentrations at or above the limit of solubility. The NOEC for this study was 3.7 µg HBCD/L. The LOEC and MATC were not determined in this study, however, they were considered to be >3.7 µg HBCD/L.

REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1400, *Fish Early-Life Stage Toxicity Test.*

2 **Organization for Economic Cooperation and Development.** 1992. *Fish Early-Life Stage Toxicity Test.* OECD Guideline for Testing of Chemicals. Guideline 210. Paris.

3 **U.S. Environmental Protection Agency.** 1986. *Standard Evaluation Procedure, Fish Early Life-Stage Test.* Office of Pesticide Programs, Hazard Evaluation Division. EPA 540/9-86-138.

4 **ASTM Standard E1241-88a.** 1988. *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish.* American Society for Testing and Materials.

5 **APHA, AWWA, WPCF.** 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

6 **Martin, J.W.** 1967. *A method of measuring lengths of juvenile salmon from photographs.* Progr. Fish-Cult. 29:238-240.

7 **West, Inc. and D. D. Gulley.** 1996. TOXSTAT, Version 3.5. Western EcoSystems Technology, Inc., Cheyenne, Wyoming.

8 **SPSS Inc.** 1988. SPSS/PC+ Version 2.0. Chicago, Illinois.

Table 1

Summary of Analytical Chemistry Data

Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Time (Day)	Replicate	Nominal Test Concentration (µg HBCD/L)	Measured Concentration (µg HBCD/L)	Mean Measured Concentration (µg HBCD/L)	Mean Percent of Nominal
0	B	Negative Control	< LOQ[1]	< LOQ	–
7	C		< LOQ		
14	D		< LOQ		
21	A		< LOQ		
28	B		< LOQ		
35	C		< LOQ		
42	D		< LOQ		
49	A		< LOQ		
56	B		< LOQ		
63	C		< LOQ		
70	D		< LOQ		
77	A		< LOQ		
84	B		< LOQ		
88	C		< LOQ		
0	B	Solvent Control	< LOQ	< LOQ	–
7	C		< LOQ		
14	D		< LOQ		
21	A		< LOQ		
28	B		< LOQ		
35	C		< LOQ		
42	D		< LOQ		
49	A		< LOQ		
56	B		< LOQ		
63	C		< LOQ		
70	D		< LOQ		
77	A		< LOQ		
84	B		<LOQ		
88	C		<LOQ		

[1]The limit of quantitation (LOQ) was 0.0400 µg a.i./L.

Table 1 (Continued)

Summary of Analytical Chemistry Data

Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Time (Day)	Replicate	Nominal Test Concentration (µg HBCD/L)	Measured Concentration (µg HBCD/L)	Mean Measured Concentration (µg HBCD/L)	Mean Percent of Nominal
0	B	0.43	0.365	0.25	58
7	C		0.351		
14	D		0.299		
21	A		0.205		
28	B		0.161		
35	C		0.298		
42	D		0.219		
49	A		0.280		
56	B		0.270		
63	C		0.225		
70	D		0.185		
77	A		0.189		
84	B		0.259		
88	C		0.230		
0	B	0.85	0.482	0.47	55
7	C		0.684		
14	D		0.647		
21	A		0.450		
28	B		0.409		
35	C		0.427		
42	D		0.562		
49	A		0.495		
56	B		0.340		
63	C		0.419		
70	D		0.389		
77	A		0.486		
84	B		0.369		
88	C		0.400		

[1]The limit of quantitation (LOQ) was 0.0400 µg a.i./L.

Table 1 (Continued)

Summary of Analytical Chemistry Data

Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Time (Day)	Replicate	Nominal Test Concentration (µg HBCD/L)	Measured Concentration (µg HBCD/L)	Mean Measured Concentration (µg HBCD/L)	Mean Percent of Nominal
0	B	1.7	0.848	0.83	49
7	C		1.03		
14	D		0.964		
21	A		0.867		
28	B		0.649		
35	C		0.816		
42	D		0.913		
49	A		0.929		
56	B		1.00		
63	C		1.01		
70	D		0.705		
77	A		0.720		
84	B		0.726		
88	C		0.385		
0	B	3.4	158	1.8	53
7	C		2.18		
14	D		2.82		
21	A		1.48		
28	B		1.28		
35	C		2.14		
42	D		1.97		
49	A		2.08		
56	B		2.60		
63	C		1.54		
70	D		1.36		
77	A		1.41		
84	B		1.11		
88	C		1.45		

[1]The limit of quantitation (LOQ) was 0.0400 µg a.i./L.

Table 1 (Continued)

Summary of Analytical Chemistry Data

Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Time (Day)	Replicate	Nominal Test Concentration (µg HBCD/L)	Measured Concentration (µg HBCD/L)	Mean Measured Concentration (µg HBCD/L)	Mean Percent of Nominal
0	B	6.8	3.39	3.7	54
7	C		3.97		
14	D		4.73		
21	A		4.77		
28	B		3.37		
35	C		3.87		
42	D		3.97		
49	A		4.54		
56	B		3.49		
63	C		3.54		
70	D		3.42		
77	A		3.38		
84	B		2.78		
88	C		2.91		

[1]The limit of quantitation (LOQ) was 0.0400 µg a.i./L.

Table 2
Temperature (°C) of Water in the Test Chambers

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Day[1]													
		0	7	15	21	28[2]	35	42	49	56	63	70	77	84	End of Test
Negative Control	A[1]	11.4	12.3	12.1	12.2	--	12.1	12.1	11.9	12.0	12.1	12.1	12.0	12.0	12.0
	B	11.4	12.3	12.1	12.2	--	12.1	12.0	11.8	11.9	12.1	12.0	12.0	12.0	12.0
	C	11.4	12.2	12.1	12.1	--	12.1	12.0	11.8	11.9	12.1	12.0	12.0	11.9	11.9
	D	11.5	12.2	12.1	12.0	--	12.1	12.0	11.8	12.0	12.1	11.9	12.0	11.9	11.9
Solvent Control	A	11.5	12.2	12.1	12.1	--	12.0	12.0	11.9	12.0	12.1	12.0	12.0	12.0	12.0
	B	11.4	12.2	12.1	12.1	--	12.0	12.0	11.9	12.0	12.1	12.0	12.0	12.0	12.0
	C	11.4	12.2	12.0	12.0	--	12.0	12.0	11.9	12.0	12.1	12.0	12.0	12.0	11.9
	D	11.4	12.2	12.0	12.0	--	12.1	12.0	11.9	12.0	12.1	12.0	12.0	12.0	12.0
0.25	A	11.4	12.2	12.0	12.0	--	12.0	12.0	11.9	12.0	12.1	12.1	12.0	12.0	12.0
	B	11.4	12.2	12.0	12.0	--	12.0	12.0	11.9	12.0	12.1	12.1	12.0	12.0	12.0
	C	11.4	12.2	12.0	12.0	--	12.0	12.0	11.9	12.0	12.1	12.0	12.0	12.0	12.9
	D	11.4	12.2	12.0	12.0	--	12.0	12.0	11.9	12.0	12.1	12.0	12.0	12.0	12.9
0.47	A	11.5	12.1	12.0	12.0	--	11.8	11.8	11.7	11.8	11.9	11.8	11.8	11.8	11.9
	B	11.4	12.1	12.0	12.0	--	11.9	11.8	11.7	11.7	11.9	11.8	11.8	11.9	11.8
	C	11.4	12.1	12.0	12.1	--	11.9	11.8	11.7	11.8	11.9	11.8	11.8	11.8	11.8
	D	11.4	12.1	12.0	12.1	--	11.9	11.9	11.8	11.8	11.9	11.8	11.9	11.8	11.9
0.83	A	11.5	12.2	12.0	12.1	--	12.1	12.0	11.9	12.0	12.1	12.0	12.0	12.0	11.9
	B	11.5	12.2	12.0	12.1	--	12.0	12.0	11.9	12.1	12.1	12.0	12.0	11.9	11.9
	C	11.5	12.2	12.1	12.1	--	12.1	12.1	11.9	12.1	12.1	12.0	12.0	11.9	12.3
	D	11.5	12.3	12.1	12.1	--	12.1	12.1	12.0	12.0	12.2	12.0	12.0	12.0	11.9
1.8	A	11.4	12.2	12.0	12.1	--	12.0	12.0	11.8	11.9	12.1	12.0	12.0	12.0	12.0
	B	11.4	12.2	12.0	12.0	--	12.0	12.0	11.8	11.9	12.1	12.0	12.0	12.0	11.9
	C	11.4	12.2	12.0	12.0	--	12.0	12.0	11.9	11.9	12.1	11.9	12.0	12.0	12.0
	D	11.4	12.2	12.0	12.0	--	12.0	12.0	11.9	12.0	12.1	11.9	12.0	12.0	12.0
3.7	A	11.4	12.1	12.0	12.0	--	12.0	11.9	11.8	11.8	12.0	11.9	11.9	11.9	11.9
	B	11.4	12.1	12.0	12.0	--	12.0	11.9	11.8	11.8	12.0	11.9	11.9	11.9	11.9
	C	11.4	12.1	12.0	12.0	--	11.9	12.0	11.8	11.8	12.0	11.9	11.9	11.9	11.9
	D	11.4	12.1	12.0	12.0	--	12.0	12.0	11.8	11.8	12.0	11.9	12.0	11.9	11.9
Fertilization Control	A	11.3	12.1	--	--	--	--	--	--	--	--	--	--	--	--
	B	11.3	12.1	--	--	--	--	--	--	--	--	--	--	--	--

[1]Temperature measured continuously during the test ranged from approximately 11.5 to 13.0°C.
[2]Temperature was inadvertently not measured on Day 28 of the test.

Table 3
Dissolved Oxygen Content (mg/L) of Water in the Test Chambers[1]

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Day																			
		0	1	2	3	4	5	6	7	15	21	28	35	42	49	56	63	70	77	84	End of Test
Negative Control	A	9.8	--	--	--	9.4	--	--	--	10.0	--	--	--	8.9	--	--	--	7.1	--	--	--
	B	--	9.1	--	--	--	10.0	--	--	--	9.4	--	--	--	8.4	--	--	--	7.0	--	--
	C	--	--	9.5	--	--	--	9.9	--	--	--	9.8	--	--	--	8.1	--	--	--	7.2	--
	D	--	--	--	9.6	--	--	--	9.9	--	--	--	8.2	--	--	--	8.0	--	--	--	7.8
Solvent Control	A	9.8	--	--	--	9.4	--	--	--	9.9	--	--	--	8.5	--	--	--	7.2	--	--	--
	B	--	9.0	--	--	--	9.9	--	--	--	9.4	--	--	--	8.2	--	--	--	6.9	--	--
	C	--	--	9.5	--	--	--	9.8	--	--	--	8.8	--	--	--	8.2	--	--	--	6.9	--
	D	--	--	--	9.4	--	--	--	9.9	--	--	--	8.0	--	--	--	8.0	--	--	--	7.6
0.25	A	9.8	--	--	--	9.4	--	--	--	9.8	--	--	--	8.6	--	--	--	7.4	--	--	--
	B	--	8.7	--	--	--	9.9	--	--	--	9.5	--	--	--	8.4	--	--	--	7.0	--	--
	C	--	--	9.2	--	--	--	9.8	--	--	--	9.0	--	--	--	8.2	--	--	--	6.8	--
	D	--	--	--	9.2	--	--	--	9.7	--	--	--	8.2	--	--	--	8.2	--	--	--	7.2
0.47	A	9.8	--	--	--	9.2	--	--	--	10.0	--	--	--	8.6	--	--	--	7.3	--	--	--
	B	--	8.8	--	--	--	9.9	--	--	--	9.4	--	--	--	8.6	--	--	--	7.4	--	--
	C	--	--	9.4	--	--	--	9.5	--	--	--	9.0	--	--	--	8.0	--	--	--	6.6	--
	D	--	--	--	9.4	--	--	--	9.8	--	--	--	8.0	--	--	--	8.0	--	--	--	6.8
0.83	A	10.0	--	--	--	9.2	--	--	--	9.9	--	--	--	8.6	--	--	--	7.0	--	--	--
	B	--	8.7	--	--	--	9.7	--	--	--	9.5	--	--	--	8.4	--	--	--	6.8	--	--
	C	--	--	9.1	--	--	--	9.4	--	--	--	8.8	--	--	--	8.1	--	--	--	6.6	--
	D	--	--	--	9.2	--	--	--	9.8	--	--	--	8.0	--	--	--	8.0	--	--	--	7.1
1.8	A	9.8	--	--	--	9.2	--	--	--	9.6	--	--	--	8.6	--	--	--	6.8	--	--	--
	B	--	8.6	--	--	--	9.8	--	--	--	9.6	--	--	--	8.2	--	--	--	7.0	--	--
	C	--	--	9.1	--	--	--	9.5	--	--	--	9.0	--	--	--	8.1	--	--	--	6.6	--
	D	--	--	--	9.2	--	--	--	9.8	--	--	--	8.0	--	--	--	8.0	--	--	--	6.8
3.7	A	9.8	--	--	--	9.2	--	--	--	9.8	--	--	--	8.6	--	--	--	6.8	--	--	--
	B	--	8.7	--	--	--	9.7	--	--	--	9.6	--	--	--	8.2	--	--	--	7.1	--	--
	C	--	--	9.2	--	--	--	9.6	--	--	--	9.4	--	--	--	8.0	--	--	--	6.7	--
	D	--	--	--	9.2	--	--	--	9.8	--	--	--	8.0	--	--	--	8.0	--	--	--	6.7
Fertilization Control	A	10.0	--	9.6	--	9.2	--	9.5	--	--	--	--	--	--	--	--	--	--	--	--	--
	B	--	8.4	--	9.2	--	9.7	--	9.9	--	--	--	--	--	--	--	--	--	--	--	--

[1]A dissolved oxygen concentration of 6.5 mg/L represents 60% saturation at 12°C in freshwater.

Table 4
pH of Water in the Test Chambers

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Day													
		0	7	15	21	28	35	42	49	56	63	70	77	84	End of Test
Negative Control	A	7.9	--	8.0	--	--	--	8.0	--	--	--	7.7	--	--	--
	B	--	--	--	7.9	--	--	--	8.0	--	--	--	7.6	--	--
	C	--	--	--	--	8.0	--	--	--	7.8	--	--	--	7.7	--
	D	--	8.1	--	--	--	8.0	--	--	--	7.9	--	--	--	7.8
Solvent Control	A	7.9	--	8.0	--	--	--	8.0	--	--	--	7.8	--	--	--
	B	--	--	--	8.0	--	--	--	8.0	--	--	--	7.7	--	--
	C	--	--	--	--	8.0	--	--	--	7.9	--	--	--	7.8	--
	D	--	8.1	--	--	--	8.0	--	--	--	7.9	--	--	--	7.9
0.25	A	7.9	--	8.0	--	--	--	7.9	--	--	--	7.8	--	--	--
	B	--	--	--	8.0	--	--	--	8.0	--	--	--	7.7	--	--
	C	--	--	--	--	8.0	--	--	--	7.9	--	--	--	7.8	--
	D	--	8.1	--	--	--	8.0	--	--	--	7.9	--	--	--	7.9
0.47	A	7.9	--	8.1	--	--	--	8.0	--	--	--	7.8	--	--	--
	B	--	--	--	8.0	--	--	--	8.1	--	--	--	7.8	--	--
	C	--	--	--	--	8.1	--	--	--	7.9	--	--	--	7.9	--
	D	--	8.1	--	--	--	8.0	--	--	--	8.0	--	--	--	7.8
0.83	A	7.9	--	8.1	--	--	--	8.0	--	--	--	7.8	--	--	--
	B	--	--	--	8.0	--	--	--	8.1	--	--	--	7.8	--	--
	C	--	--	--	--	8.1	--	--	--	7.9	--	--	--	7.9	--
	D	--	8.1	--	--	--	8.0	--	--	--	8.0	--	--	--	7.9
1.8	A	8.0	--	8.1	--	--	--	8.0	--	--	--	7.8	--	--	--
	B	--	--	--	8.0	--	--	--	8.1	--	--	--	7.8	--	--
	C	--	--	--	--	8.1	--	--	--	7.9	--	--	--	8.0	--
	D	--	8.1	--	--	--	8.0	--	--	--	8.0	--	--	--	7.8
3.7	A	8.0	--	8.1	--	--	--	8.0	--	--	--	7.8	--	--	--
	B	--	--	--	8.0	--	--	--	8.1	--	--	--	7.8	--	--
	C	--	--	--	--	8.1	--	--	--	7.9	--	--	--	8.0	--
	D	--	8.1	--	--	--	8.0	--	--	--	8.0	--	--	--	7.9
Fertilization Control	A	7.9	--	--	--	--	--	--	--	--	--	--	--	--	--
	B	--	8.1	--	--	--	--	--	--	--	--	--	--	--	--

Table 5

Conductivity, Hardness, and Alkalinity of Water in the
Negative Control and One Treatment Group

Negative Control

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

	Day													
Parameter	0	7	14	21	28	35	42	49	56	63	70	77	84	End of Test
Conductivity (μmhos/cm)	280	305	290	280	290	305	300	300	280	290	285	290	295	290
Hardness (mg/L as $CaC0_3$)	136	124	120	128	132	120	134	136	136	136	136	136	136	124
Alkalinity (mg/L as $CaC0_3$)	182	182	182	184	174	182	180	180	180	188	179	182	184	180
Replicate	A	D	A	B	C	D	A	B	C	D	A	B	C	D

Table 5 (Continued)

Conductivity, Hardness, and Alkalinity of Water in the
Negative Control and One Treatment Group

3.7 µg HBCD/L

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

	Day													
Parameter	0	7	14	21	28	35	42	49	56	63	70	77	84	End of Test
Conductivity (μmhos/cm)	280	305	280	280	280	305	290	300	270	280	285	285	290	285
Hardness (mg/L as $CaC0_3$)	132	120	112	132	132	136	136	136	136	136	136	132	136	130
Alkalinity (mg/L as $CaC0_3$)	182	182	184	186	174	180	182	180	180	186	178	179	184	180
Replicate	A	D	A	B	C	D	A	B	C	D	A	B	C	D

Table 6

Egg Viability (Percent Fertilization)[1]

Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Test Chamber	Total Number of Eggs	Number of Viable Eggs	Mean Percent Viability
Fertilization Control Cup 1, Replicate A	30	29	99.2
Fertilization Control Cup 2, Replicate A	30	30	
Fertilization Control Cup 1, Replicate B	30	30	
Fertilization Control Cup 2, Replicate B	30	30	

[1] Day 11 of the test.

Table 7

Cumulative Embryo Mortality and Hatching Success

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Number of Eggs Exposed	Cumulative Embryo Mortality (Day)[1]										
			1	2	3	4	5	6	7	8	9	10	11
Negative Control	A	30	0	0	0	0	0	0	0	0	0	0	0
	B	32	0	0	0	0	0	0	0	0	0	0	0
	C	30	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	A	30	0	0	0	0	0	0	0	1	1	1	1
	B	30	0	0	0	0	0	0	0	0	0	1	1
	C	30	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	0	0	0	0	0	0	1	1
0.25	A	30	1	1	1	1	1	1	1	1	1	1	1
	B	30	0	0	0	0	0	0	0	0	0	1	1
	C	30	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	0	0	0	0	0	0	0	0
0.47	A	30	0	0	0	0	0	0	0	0	0	0	0
	B	30	0	0	0	0	0	0	0	0	0	0	0
	C	30	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	0	0	0	0	0	0	0	0
0.83	A	30	0	0	0	0	0	0	0	0	0	0	0
	B	30	0	0	0	0	0	0	0	0	0	0	0
	C	30	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	0	0	0	0	0	0	0	0
1.8	A	30	0	0	0	0	0	0	0	0	0	0	0
	B	30	0	0	0	0	0	0	0	0	0	0	0
	C	30	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	0	0	0	0	0	0	0	0
3.7	A	30	0	0	0	0	0	0	0	0	0	0	0
	B	30	0	0	0	0	0	0	0	0	0	0	0
	C	31	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	0	0	0	0	0	0	0	0

[1] Number in parentheses equals number hatched.

Table 7 (Continued)
Cumulative Embryo Mortality and Hatching Success

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Number of Eggs Exposed	Cumulative Embryo Mortality (Day)[1]										
			12	13	14	15	16	17	18	19	20	21	22
Negative Control	A	30	0	1	3	10	10	12	12	12	12	12	12
	B	32	0	1	3	3	3	3	3	3	3	3	3
	C	30	0	1	2	2	2	2	2	2	2	3	3
	D	30	0	1	1	1	1	3	3	3	4	4	4
Solvent Control	A	30	1	2	2	2	2	2	2	3	3	3	3
	B	30	1	1	1	1	1	3	4	4	4	4	4
	C	30	0	0	2	2	2	5	5	5	5	5	5
	D	30	1	1	2	2	2	2	2	2	2	2	2
0.25	A	30	1	2	2	2	2	4	4	4	4	4	4
	B	30	1	1	1	1	1	2	2	3	3	3	3
	C	30	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	0	0	1	1	1	1	1	1
0.47	A	30	0	0	0	0	0	0	1	1	1	1	1
	B	30	0	1	1	1	1	1	1	1	1	1	1
	C	30	0	1	1	1	1	3	3	4	4	4	4
	D	30	1	1	3	4	4	5	5	5	5	5	5
0.83	A	30	1	3	3	4	4	4	4	4	4	4	4
	B	30	0	0	2	2	2	2	2	2	2	2	2
	C	30	0	0	1	1	1	1	1	1	1	1	1
	D	30	0	0	0	0	0	1	1	1	1	1	1
1.8	A	30	0	1	1	1	1	2	2	2	2	2	2
	B	30	0	3	3	4	4	6	7	7	7	7	7
	C	30	1	1	1	1	1	2	3	3	3	3	3
	D	30	0	0	0	0	0	0	0	1	1	1	1
3.7	A	30	0	2	2	2	2	7	7	7	7	7	7
	B	30	0	0	2	3	3	3	3	3	3	3	3
	C	31	0	0	0	0	0	0	0	0	0	0	0
	D	30	0	0	0	2	2	2	2	2	2	2	2

[1] Number in parentheses equals number hatched.

Table 7 (Continued)
Cumulative Embryo Mortality and Hatching Success

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Number of Eggs Exposed	Cumulative Embryo Mortality (Day)[1]											Percent Hatching Success
			23	24	25	26	27[2]	28	29	30	31	32	33	
Negative Control	A	30	13	13	13	13	14(16)	14(16)	14(16)	14(16)	14(16)	14(16)	14(16)	75
	B	32	3	3	3	3	3(27)	3(28)	3(28)	3(29)	3(29)	3(29)	3(29)	
	C	30	3	3	3	3(1)	7(21)	7(22)	7(22)	7(23)	7(23)	7(23)	7(23)	
	D	30	4	4	4	4	6(22)	6(23)	6(23)	6(24)	6(24)	6(24)	6(24)	
Solvent Control	A	30	4	4	4	4	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	85
	B	30	4	4	4	4	4(25)	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	
	C	30	5	5	5	5	6(24)	6(24)	6(24)	6(24)	6(24)	6(24)	6(24)	
	D	30	2	2	2	2	2(24)	2(25)	2(25)	2(26)	2(26)	4(26)	4(26)	
0.25	A	30	4	4	4	4	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	91
	B	30	3	3	3	3	3(25)	3(26)	4(26)	4(26)	4(26)	4(26)	4(26)	
	C	30	0	0	0	0	0(30)	0(30)	0(30)	0(30)	0(30)	0(30)	0(30)	
	D	30	1	1	1	1(1)	1(28)	1(28)	2(27)	2(27)	2(27)	3(27)	3(27)	
0.47	A	30	1	1	1	1(1)	1(27)	1(28)	1(28)	1(28)	1(28)	2(28)	2(28)	89
	B	30	1	1	1	1	1(28)	1(29)	1(29)	1(29)	1(29)	1(29)	1(29)	
	C	30	4	4	4	4	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	
	D	30	5	5	5	5	5(22)	6(24)	6(24)	6(24)	6(24)	6(24)	6(24)	
0.83	A	30	4	4	4	4	5(19)	5(21)	5(23)	5(23)	5(24)	6(24)	6(24)	89
	B	30	2	2	2	2(2)	2(27)	2(27)	2(27)	2(27)	2(27)	2(27)	2(28)	
	C	30	1	1	1	1	2(26)	2(26)	2(26)	2(26)	2(26)	3(26)	3(27)	
	D	30	1(1)	1(1)	1(1)	1(4)	1(25)	1(26)	1(27)	1(27)	1(27)	2(27)	2(28)	
1.8	A	30	2	2	2	2(1)	2(26)	2(27)	3(26)	4(26)	4(26)	4(26)	4(26)	83
	B	30	7	7	7	7	8(21)	8(22)	8(22)	8(22)	8(22)	8(22)	8(22)	
	C	30	3	3	3	3	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	4(26)	
	D	30	1	1	4	4	4(25)	4(25)	4(25)	5(25)	5(25)	5(25)	5(25)	
3.7	A	30	7	7	7	7	8(20)	9(20)	9(20)	9(20)	9(20)	9(20)	9(21)	84
	B	30	3	3	3	3	4(24)	5(24)	5(24)	5(24)	5(24)	5(24)	5(25)	
	C	31	0	0	0	0(1)	0(27)	0(29)	0(29)	0(29)	0(29)	2(29)	2(29)	
	D	30	2	2	2	2	2(26)	2(27)	2(27)	3(27)	3(27)	3(27)	3(27)	

[1] Number in parentheses equals number hatched.
[2] Day 27 = Day 0 Post-Hatch. All larvae were released into the test chambers on this day.

Table 8

Time to Swim-Up

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Cumulative Number Swimming Up/Number Alive at Each Post-Hatch Day									
	Day 13[1]	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22[2]
Negative Control	3/90	15/89	51/89	67/89	77/89	62/89	66/89	72/89	79/89	86/89
Solvent Control	2/100	22/100	71/100	87/100	93/100	81/100	83/100	83/100	84/100	96/100
0.25	4/108	27/108	73/108	86/108	101/108	92/108	92/108	93/108	96/108	105/108
0.47	0/107	24/107	69/105	87/105	94/105	86/105	87/105	89/105	91/105	104/105
0.83	1/106	25/106	70/106	83/106	94/106	84/106	85/106	85/106	89/106	103/106
1.8	2/99	28/99	65/99	80/98	90/98	77/98	82/98	82/98	84/98	97/98
3.7	0/100	14/99	40/99	72/99	85/99	75/99	76/99	76/99	80/99	97/99

[1] Day 13 post-hatch equals Day 40 of the test.
[2] On Day 22 post-hatch, all treatments impartially thinned to 60 fish per treatment level.

Table 9
Survival of Larvae from the Beginning of the Post-Hatching Period to Thinning

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Total Number of Larvae Hatched	Days Post-Hatch																						Mean % Survival
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Negative Control	A	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	97
	B	29	28	28	29	29	29	29	29	29	29	29	29	29	29	28	28	28	28	28	28	28	28	28	
	C	23	22	22	23	23	23	23	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	
	D	24	23	23	24	24	24	24	24	24	23	23	23	23	23	23	23	23	23	23	23	23	23	23	
Solvent Control	A	26	26	26	26	26	26	26	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	98
	B	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	
	C	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	
	D	26	25	25	26	26	26	26	26	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
0.25	A	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	99
	B	26	26	26	26	26	26	26	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
	C	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	
	D	27	28	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	
0.47	A	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	98
	B	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	
	C	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	24	24	24	24	24	24	24	24	
	D	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	
0.83	A	24	21	23	23	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	99
	B	28	27	27	27	27	27	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	
	C	27	26	26	26	26	26	27	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	
	D	28	26	27	27	27	27	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	
1.8	A	26	27	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	99
	B	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	21	21	21	21	21	21	21	
	C	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	
	D	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
3.7	A	21	20	20	20	20	20	21	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	97
	B	25	24	24	24	24	24	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
	C	29	29	29	29	29	29	29	29	29	29	29	29	29	29	28	28	28	28	28	28	28	28	28	
	D	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	

Table 10

Survival of Larvae from Day 22 to Day 61 Post-Hatch

Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Initial Number of Larvae	Days Post-Hatch							% Survival	Mean % Survival
			22	28	35	42	49	56	61		
Negative Control	A	15	15	15	15	15	15	15	15	100	98
	B	15	15	15	15	15	15	15	14	93	
	C	15	15	15	15	15	15	15	15	100	
	D	15	15	15	15	15	15	15	15	100	
Solvent Control	A	15	15	15	15	15	15	15	15	100	98
	B	15	15	15	15	15	15	15	15	100	
	C	15	15	15	14	14	14	14	14	93	
	D	15	15	15	15	14*	14	14	14	100	
0.25	A	15	15	15	15	15	15	15	15	100	100
	B	15	15	15	15	15	15	15	15	100	
	C	15	15	15	15	15	15	15	15	100	
	D	15	15	15	15	15	15	15	15	100	
0.47	A	15	15	15	15	15	15	15	13	87	97
	B	15	15	15	15	15	15	15	15	100	
	C	15	15	15	15	15	15	15	15	100	
	D	15	15	15	15	15	15	14**	14	100	
0.83	A	15	15	15	15	14	14	14	14	93	98
	B	15	15	15	15	15	15	15	15	100	
	C	15	15	15	15	15	15	15	15	100	
	D	15	15	15	15	15	15	15	15	100	
1.8	A	15	15	15	15	15	15	15	15	100	100
	B	15	15	15	15	15	15	15	15	100	
	C	15	15	15	15	15	15	15	15	100	
	D	15	15	15	15	15	15	15	15	100	
3.7	A	15	15	15	15	15	15	15	15	100	100
	B	15	15	15	15	15	15	15	15	100	
	C	15	15	15	15	15	15	15	15	100	
	D	15	15	15	15	15	15	15	15	100	

*One fish inadvertently killed during siphoning on Day 66 (Day 39 post-hatch). This fish was excluded in calculation of the survival percentage for the replicate.

**One fish jumped out of the holding bucket during cleaning of the test chambers on Day 82 (Day 55 post-hatch). This fish was excluded in calculation of the survival percentage for the replicate.

Table 11

Most Frequent Behavioral and Appearance Characteristics

Sponsor:	American hemistry Council's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Mean Measured Concentration (µg HBCD/L)	Week Post-Hatch – Most Frequent Observations								
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Negative Control	AN	AN	AN	AN	AN	AN	AN	AN	AN
Solvent Control	AN	AN	AN	AN	AN	AN	AN	AN	AN
0.25	AN	AN	AN	AN	AN	AN	AN	AN	AN
0.47	AN	AN	AN	AN	AN	AN	AN	AN	AN
0.83	AN	AN	AN	AN	AN	AN	AN	AN	AN
1.8	AN	AN	AN	AN	AN	AN	AN	AN	AN
3.7	AN	AN	AN	AN	AN	AN	AN	AN	AN

Behavioral and Appearance Codes:
AN = Appear normal

Table 12
Mean Total Length at Day 29 and Day 61 Post-Hatch

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Day 29 Post-Hatch Mean Total Length (mm)	Day 29 Post-Hatch Overall Mean (± SD)[1]	Day 61 Post-Hatch Mean Total Length (mm)	Day 61 Post-Hatch Overall Mean (± SD)[1]
Negative Control	A	30.7	30.8 (± 0.379)	50.3	50.1 (± 0.208)
	B	31.3		50.1	
	C	30.5		50.0	
	D	30.5		49.8	
Solvent Control	A	31.7	30.9 (± 0.714)	49.4	49.9 (± 0.716)
	B	31.1		50.3	
	C	30.0		50.7	
	D	30.7		49.2	
0.25	A	30.0	30.1 (± 0.386)	50.2	49.6 (± 0.723)
	B	29.9		49.0	
	C	30.7		50.3	
	D	29.9		49.0	
0.47	A	30.1	30.1 (± 0.450)	49.7	49.5 (± 0.171)
	B	30.5		49.3	
	C	29.5		49.6	
	D	30.4		49.5	
0.83	A	30.2	30.6 (± 0.479)	49.6	49.6 (± 0.0957)
	B	31.2		49.5	
	C	30.7		49.7	
	D	30.2		49.5	
1.8	A	30.7	30.8 (± 0.929)	49.3	49.2 (± 0.727)
	B	29.5		48.2	
	C	31.7		49.9	
	D	31.1		49.5	
3.7	A	31.2	31.1 (± 0.424)	48.7	49.6 (±0.656)
	B	31.5		50.2	
	C	31.2		49.4	
	D	30.5		49.9	

Table 13

Mean Wet Weight and Dry Weight at 61 Days Post-Hatch

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Mean Wet Weight (g)	Overall Mean Wet Weight (± SD) (g)	Mean Dry Weight (g)	Overall Mean Dry Weight (± SD) (g)
Negative Control	A	1.0871	1.0999 (± 0.0127)	0.2350	0.2381 (± 0.0026)
	B	1.1086		0.2405	
	C	1.1129		0.2393	
	D	1.0912		0.2373	
Solvent Control	A	1.1235	1.1330 (± 0.0257)	0.2414	0.2419 (± 0.0071)
	B	1.1393		0.2479	
	C	1.1649		0.2462	
	D	1.1042		0.2321	
0.25	A	1.1757	1.1151 (± 0.0490)	0.2531	0.2413 (± 0.0101)
	B	1.0684		0.2293	
	C	1.1333		0.2449	
	D	1.0830		0.2379	
0.47	A	1.1209	1.1207 (± 0.0327)	0.2392	0.2379 (± 0.0090)
	B	1.0913		0.2330	
	C	1.1662		0.2500	
	D	1.1042		0.2294	
0.83	A	1.1304	1.0789 (± 0.0403)	0.2328	0.2295 (± 0.0066)
	B	1.0608		0.2280	
	C	1.0881		0.2361	
	D	1.0364		0.2210	
1.8	A	1.0733	1.0728 (± 0.0582)	0.2358	0.2294 (± 0.0154)
	B	0.9919		0.2069	
	C	1.0994		0.2336	
	D	1.1266		0.2414	
3.7	A	1.0568	1.0961 (±0.0325)	0.2287	0.2355 (± 0.0064)
	B	1.1363		0.2441	
	C	1.0970		0.2349	
	D	1.0943		0.2344	

Appendix 1

Protocol, Protocol Amendments and Protocol Deviations

PROTOCOL

HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.1400

OECD Guideline 210

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

August 1, 2000

Wildlife International, Ltd.

- 2 -

HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

SPONSOR:	American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Kurt Drottar Senior Aquatic Biologist
LABORATORY MANAGEMENT:	Henry O. Krueger, Ph.D. Director of Aquatic Toxicology & Non-Target Plants

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: _____ Experimental Termination Date: _____

Project No.: 439A-112

Test Concentrations: _____

Test Substance No.: _____ Reference Substance No. (if applicable): _____

PROTOCOL APPROVAL

_____ STUDY DIRECTOR	8/8/00 DATE
_____ LABORATORY MANAGEMENT	8/8/00 DATE
Wendy K. Sherman SPONSOR'S REPRESENTATIVE	8/7/00 DATE

Wildlife International, Ltd.

INTRODUCTION

Wildlife International, Ltd. will conduct an early life-stage toxicity test with the rainbow trout (*Oncorhynchus mykiss*) for the Sponsor at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.1400 (1); OECD Guideline for Testing of Chemicals 210: *Fish, Early-Life Stage Toxicity Test* (2); *Standard Evaluation Procedure, Fish Early Life-Stage Test* (3); and *ASTM Standard E1241-88a Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish* (4). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to determine the effects of HBCD on the time to hatch, hatching success, time to swim-up, survival, and growth of rainbow trout (*Oncorhynchus mykiss*) during early life-stage development.

EXPERIMENTAL DESIGN

Rainbow trout embryos will be exposed to a series of at least five test concentrations, a negative (dilution water) control and, if necessary, a solvent control. Target concentrations will not exceed 120 mg/L or the solubility limit of the test substance in water (whichever is lower). Nominal test concentrations will be selected in consultation with the Sponsor, and will be based upon information such as the results of exploratory range-finding toxicity data. Each test concentration will be at least 50% of the next higher treatment level, unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate.

The test will begin when groups of newly-fertilized embryos are placed in incubation cups and exposed to test solution. Two incubation cups, each containing 15 embryos, will be placed in each of four replicate test chambers per treatment (total of 120 embryos per treatment). An additional thirty embryos will be held in each of four extra incubation cups in dilution water and will be sacrificed between Days 10 and 12 to evaluate fertilization success. The embryo exposure period will last approximately 26-32 days. After hatching, larvae will be released from the incubation cups into the test chambers where exposure will continue. Once >90% of the control group reaches the swim-up stage, the number of larvae in all replicates

Wildlife International, Ltd.

will be reduced to 15 to prevent overcrowding and exposure will continue for at least 60 days post-hatch. Length will be assessed approximately 30 days after hatching using photographic techniques, and at the end of the 60-day larval exposure period, the wet weight, dry weight and the total length of each surviving fish will be determined.

Observations of the effects of the test substance on hatching success, time to hatch, time for larvae to swim up, and post-hatch growth, and survival will be used to calculate the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The NOEC and LOEC will be used to calculate the maximum acceptable toxicant concentration (MATC).

MATERIALS AND METHODS

Test Substance

The test substance consisted of a composite of HBCD samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International Ltd. Identification Number
Great Lakes Chemical Corporation	Not Given	June 19, 1998	4515
Eurobrom b.v.	971201	June 25, 1998	4520
Albemarle Corporation	33449-15X	June 29, 1998	4521

The composite test substance was assigned Wildlife International Ltd. identification number 4615 and was stored under ambient conditions. A subsample of the composite test substance was shipped to Albemarle Corporation for characterization and purity analyses. The results of the analyses indicated the composite test substance was homogeneous and contained the following components:

Alpha Isomer	6.4%
Beta Isomer	4.5%
Gamma Isomer	79.1%

The conclusion of the characterization was that the test article was HBCD with a purity of 90.0% HBCD isomers. The test substance was stored at room temperature.

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Preparation of Test Concentrations

The test substance will be administered to the test organism in water. This route of administration was selected because it represents the most likely route of exposure to aquatic organisms.

The test substance will be mixed directly with dilution water or may be first mixed with a solvent. If a solvent is used, the test substance will be dissolved in the solvent to form a stock solution that will subsequently be added to the dilution water. Reverse osmosis water will be the solvent of choice, although dimethyl formamide, triethylene glycol, methanol, ethanol, or acetone may be used. If an organic solvent is required, a solvent control will be included in the experimental design along with a negative (dilution water) control. The concentration of the organic solvent will not exceed 0.1 mL/L, when possible. The solvent concentration in the solvent control will be equal to that in the chambers containing the test substance.

Test Organism

Newly-fertilized embryos of the rainbow trout (*Oncorhynchus mykiss*) will be used in this test. This species is representative of an important group of organisms and was selected for use in the test based upon past use and ease of handling in the laboratory. Unfertilized eggs and sperm will be obtained from a suitable commercial supplier. Gametes from a minimum of 2-3 spawns will be used in the test. Fertilization will take place at Wildlife International, Ltd. and the test will be initiated within 24 hours of fertilization.

Once greater than 90% of control larvae have reached swim-up stage, feeding will begin using salmon-starter mash. Swim-up larvae will be fed 3 times per day during the first 7 days. Thereafter, they will be fed salmon-starter mash 3 times daily on weekdays and at least 2 times daily on weekends and holidays until the test is terminated. To ensure that the feeding rate per fish remains constant, rations will be adjusted on a weekly basis to account for losses due to mortality. Fish will not be fed at least 48 hours prior to the termination of the test to allow the fish to clear their digestive tracts before measurements of weight are made. Embryos and larval fish will be handled as little as possible, but when handling is necessary, it will be done carefully, gently and quickly.

Specifications for acceptable levels of contaminants in fish diets have not been established. However, there are no known levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the test.

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Loading, defined as the total wet weight of fish per liter of test solution, will not exceed 0.5 grams of fish per liter of solution that passes through a chamber in 24 hours. Instantaneous loading will not exceed 5 grams of fish per liter of test solution present in the test chamber at any given time.

Dilution Water

Water used for the holding and testing of rainbow trout will be obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The water will be passed through a sand filter and pumped into a 37,800-L storage tank where the water will be aerated with spray nozzles. Prior to use the water will be filtered to 0.45 μm in order to remove fine particles and then passed through a UV sterilizer in order to remove microorganisms. Water used for holding and testing is characterized as moderately hard. Typical values for hardness, alkalinity, pH and specific conductance are approximately:

Hardness, mg/L as $CaCO_3$	145
Alkalinity, mg/L as $CaCO_3$	190
pH	8.1
Specific Conductance, μmhos/cm	330

Hardness, alkalinity, pH and specific conductance will be measured weekly to monitor the consistency of the well water. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and results of the most recent GLP-compliant analyses will be summarized in the final report.

Test Apparatus

A continuous-flow diluter will be used to provide each concentration of the test substance, a negative (dilution water) control, and a solvent control, when necessary. A syringe pump, peristaltic pump, or a similar device will be used to deliver the test substance to mixing chambers where the test substance will be mixed with dilution water. The flow of dilution water into each mixing chamber will be controlled using rotameters. The rotameters will be calibrated prior to test initiation and at approximately weekly intervals thereafter. After mixing, test solutions will be split to each replicate chamber. The proportion of water split to each replicate will be checked prior to the test and at approximately weekly intervals thereafter to ensure that these flow rates vary by no more than ± 10% of the mean flow rate of the four replicates.

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The diluter will be adjusted so that each test chamber receives at least 5 volume additions of test solution every 24 hours. Peristaltic pumps, if used, will be calibrated before each study and at approximately weekly intervals thereafter. Syringe pumps, if used, will be calibrated prior to beginning the test. The delivery of test substance to test chambers will begin at least 4 hours prior to the test in order to establish equilibrium concentrations of the test substance. The general operation of the diluter will be checked visually at least two times per day during the test and at least once at the beginning and end of the test.

Embryo incubation cups will be constructed from glass cylinders approximately 50 mm in diameter, with 425-μm nylon or Teflon® screen attached to the bottom using silicone sealant. Test chambers will be 9-L glass aquaria filled with approximately 7 L of water. Test chambers will be positioned in a temperature-controlled environmental chamber to maintain a temperature of 12 ± 1°C. Test chambers will be labeled with project number, replicate and test concentration.

Environmental Conditions

The rainbow trout larvae will be kept in darkness (except during inspections) until one week after hatching. Thereafter, during the test, the test organisms will be kept under subdued lighting. Fluorescent tubes that emit wavelengths similar to natural sunlight (e.g., Colortone® 50) will be controlled by an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. A 30-minute transition of low light intensity will be provided when lights go on and off to avoid sudden changes in light intensity. Light intensity will be measured when the light/dark photoperiod is initiated with a SPER Scientific Ltd. light meter or equivalent.

The test will be conducted at a target temperature of 12 ± 1°C. Temperature will be monitored and recorded continuously during the entire test in one control replicate using a Fulscope ER/C Recorder (1900 J Series Model A) or equivalent. Recorder measurements will be verified with a liquid-in-glass thermometer prior to test initiation and verified/calibrated at weekly intervals thereafter. The temperature in each test chamber also will be measured using a liquid-in-glass thermometer at the beginning of the test, at weekly intervals during the test, and at the end of the test.

Dissolved oxygen will be measured in alternate replicates of each treatment and control group at the beginning of the test, daily during the first 7 days of the test, at weekly intervals during the test and at test termination using a Yellow Springs Instrument Model 51B dissolved oxygen meter, or equivalent. In the

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event that dissolved oxygen levels fall below 60% saturation, dissolved oxygen measurements will be made in every test chamber and appropriate actions will be taken after consultation with the Sponsor. Measurements of pH will be made in alternate replicates of each treatment and control group at test initiation, at weekly intervals during the test and at test termination using a Fisher Accumet Model 915 pH meter, or equivalent. If a treatment level reaches 100% mortality, temperature, dissolved oxygen and pH measurements will be taken at that time then discontinued.

Hardness, alkalinity, and specific conductance will be measured in the dilution water and in one treatment level at test initiation, at weekly intervals during the test and at test termination. Hardness and alkalinity measurements will be made by titration using procedures based on methods in *Standard Methods for the Examination of Water and Wastewater* (5). Specific conductance will be measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter, or equivalent.

Procedures

Prior to test initiation, embryo incubation cups will be placed in glass beakers containing dilution water within 3°C of the test temperature. In order to control bias, one, two or three embryos will be indiscriminately distributed among the cups until each contains 15 embryos. No other potential sources of bias are expected to affect the results of the study. Two incubation cups will be placed in each replicate test chamber to achieve a total of 30 embryos per replicate and 120 embryos per treatment. Four additional cups, each containing 30 embryos, will be held in test chambers containing dilution water and will be sacrificed between Days 10 and 12 to evaluate egg viability. Incubation cups will be attached to a rocker arm and suspended in the water column of the test chambers. The reciprocating motion of the rocker arm (approximately 2 rpm) will facilitate circulation of test solution around the embryos during the incubation period.

Dead embryos and/or eggs with fungus will be counted and removed approximately daily to avoid contaminating viable embryos. Any unhatched embryos will be kept in the egg cups until they have hatched or until death of the embryo occurs. When hatching has reached >95% in the control group, larvae will be released to their respective test chambers and the post-hatch period will begin.

Once >90% of the control group reaches swim-up stage, the number of larvae in each replicate will be thinned to 15, unless the number surviving is less than 15, and exposure will continue for at least 60 days

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post-hatch. The test will be repeated if the percentage of embryos in the control group that hatches successfully is less than 66% or if control post-hatch survival is less than 70%.

After hatching, observations of mortality, unusual behavior, and overall appearance of the fry will be made daily during the test. At approximately 30 days post-hatch, the total lengths of the fry will be measured using the photometric method of Martin (6). At the conclusion of the test, the wet weight, dry weight and total length of each surviving fish will be measured.

Biological Measurements

Data on time to hatch, hatching success, time to swim-up, survival, and growth (wet weight, dry weight and total length) will be collected during the test. Daily observations of fry mortality, unusual behavior, and overall appearance will be made during the 60-day post hatch growth period. At test termination, all surviving fish will be retained for measurements of wet weight, dry weight, and total length.

Sampling for Analytical Measurements

In the definitive test, water samples will be collected from all levels prior to exposure to measure concentrations of the test substance in water. Samples will be collected from alternating replicates (A, B, C or D) of all levels at test initiation, at weekly intervals throughout the test, and at test termination. This will result in approximately 112 verification samples and approximately 64 QC samples. In the event that 100% mortality occurs in any treatment, then sampling of that treatment will terminate following the next sampling interval. Samples will be collected at mid-depth from each test chamber, analyzed immediately or placed in an appropriate storage container (e.g., glass or polypropylene bottle) and stored under refrigeration until analyzed. At the discretion of the Study Director, water samples also will be collected from at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

Analytical Measurements

Chemical analysis of the samples will be performed by Wildlife International, Ltd. using either HPLC with UV detection or liquid chromatography/mass spectrometry (LC/MS). The alpha, beta and gamma moieties of HBCD in each sample will be quantified. A summary of the analytical method will be documented in the raw data and described in the final report.

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Data Analyses

Hatching success, survival of juvenile fish and time to swim-up data will be evaluated using 2 X 2 contingency tables or a similar statistical comparison test to identify those treatments statistically different from the control group.

Total length, wet weight and dry weight of surviving fish will be evaluated for normality and homogeneity of variances. Transformations will be used when necessary to correct for non-normality or heterogeneity of variances. If a solvent control group is used in addition to a negative control group, these two groups will be compared. If no statistical differences are found, then the data of the two control groups may be pooled. If statistical differences are found, then either the negative or solvent control groups will be used to evaluate the treatment-related effects.

When the growth data are considered to be normal with homogeneous variances, an analysis of variance (ANOVA) will be used to determine whether or not statistical differences exist among the experimental groups. If statistical differences are found, then a means comparison test (e.g., Dunnett's test, the Bonferroni t-test, or an alternative test) will be used to identify those treatments differing from the control group(s). When transformations fail to correct for non-normality or heterogeneous variances, then non-parametric analyses will be used to evaluate treatment-related effects. The NOEC, the LOEC and the MATC will be determined using the results of the statistical analyses.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated at Wildlife International, Ltd. will include, but not be limited to:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. History of the test organism.
5. Weight and length measurements.
6. Stock solution calculation and preparation.
7. Observations made during the test.
8. Water chemistry calculations (e.g., hardness and alkalinity).

Wildlife International, Ltd.

9. If applicable, the methods used to analyze test substance concentrations and the results of analytical measurements.
10. Statistical calculations.
11. Test conditions and physical/chemical measurements.
12. Calculation and preparation of test concentrations.
13. Copy of final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report will include, but not be limited to, the following:

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed. It is the responsibility of the Sponsor to provide the final date that data are recorded for chemistry, pathology and/or supporting evaluations that may be generated at other laboratories.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures as stated in the approved protocol, including any changes in the original protocol.
5. The test substance identification, including name, chemical abstract number or code number, strength, purity, composition, and other characteristics provided by the Sponsor.
6. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor.
7. A description of the methods used to conduct the test.
8. A description of the test organisms, including the source of the test organisms, scientific name, age, life stage, means and ranges of weights and lengths, observed diseases and treatments.
9. A description of the preparation of the test solutions, the methods used to allocate organisms to test chambers and begin the test, the number of organisms and chambers per treatment, and the duration of the test.
10. A description of circumstances that may have affected the quality or integrity of the data.
11. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.

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12. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological and analytical chemistry data, and a statement of the conclusions drawn from the analyses.
13. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
14. Statistical methods employed for analyzing the data.
15. The location where raw data and final report are to be stored.
16. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
17. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed and dated by the Study Director.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

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REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1400, *Fish Early-Life Stage Toxicity Test.*

2 **Organization for Economic Cooperation and Development.** 1992. *Fish Early-Life Stage Toxicity Test.* OECD Guideline for Testing of Chemicals. Guideline 210. Paris.

3 **United States Environmental Protection Agency.** 1986. *Standard Evaluation Procedure, Fish Early Life-Stage Test.* Office of Pesticide Programs, Hazard Evaluation Division. EPA 540/9-86-138.

4 **ASTM Standard E1241-88a.** 1994. *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish.* American Society for Testing and Materials.

5 **APHA, AWWA, WPCF.** 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

6 **Martin, J.W.** 1967. *A method of measuring lengths of juvenile salmon from photographs.* Progr. Fish-Cult. 29:238-240.

Wildlife International, Ltd.

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APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): HBCD

Test Substance Sample Code or Batch Number: Wildlife International Ltd. Identification No. 4615

Test Substance Purity (% Active Ingredient): 90.0% - HBCD Expiration Date: _____

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? X Yes _____ No

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd.

Ambient

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? _____ Yes _____ No

Other pertinent stability information:

IV. Test Concentrations:

_____ Adjust test concentration to 100% a.i. based upon the purity (%) given above.

x Do not adjust test concentration to 100% a.i. Test the material AS IS.

V. Toxicity Information:

Mammalian: Rat LD50 _____ Mouse LD50: _____

Aquatic:	Invertebrate Toxicity (EC/LC50)	Fish Toxicity (LC50)
	_____	_____
	_____	_____

Other Toxicity Information (including findings of chronic and subchronic tests):

VI. Classification of the Compound:

_____ Insecticide _____ Herbicide _____ Fungicide

_____ Microbial Agent _____ Economic Poison

Other: _____

PROJECT NO.: 439A-112
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WILDLIFE INTERNATIONAL LTD.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/080100/RBT-ELS/SUB439 **AMENDMENT NO.:** 1

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-112

EFFECTIVE DATE: August 21, 2000

AMENDMENT: Page 2

Add: Experimental Start Date: 8/24/00
Experimental Termination Date: 11/22/00
Test Concentrations: Negative Control, Solvent Control (0.1 mL acetone/L), 0.43, 0.85, 1.7, 3.4 and 6.8 µg HBCD/L

REASON: The above information was not known when the protocol was signed by the Study Director.

STUDY DIRECTOR — DATE 8/25/00

LABORATORY MANAGEMENT — DATE 8/25/00

Wendy K. Sherman
SPONSOR'S REPRESENTATIVE — DATE 9/6/00

KRD:\439A112\pral

Reviewed by QA SLC 8-24-00

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WILDLIFE INTERNATIONAL LTD.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/080100/RBT-ELS/SUB439 **AMENDMENT NO.:** 2

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-112

EFFECTIVE DATE: August 8, 2000

AMENDMENT: Page 2

Add: Reference Substance Number: 5204A, 5204B, 5204C

REASON: To add the analytical standards to the protocol.

STUDY DIRECTOR — DATE 10/3/00

LABORATORY MANAGEMENT — DATE 10/3/00

Wendy K. Shuma
SPONSOR'S REPRESENTATIVE — DATE 10/13/00

KRD:\439A\12\pra2

Reviewed by QA SLC 10-2-00

PROJECT NO.: 439A-112
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WILDLIFE INTERNATIONAL LTD.

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/080100/RBT-ELS/SUB439 **DEVIATION NO.:** 1

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-112

DATE OF DEFACTO DEVIATION: August 24, 2000

DEVIATION: The protocol states that two incubation cups will be placed in each replicate test chamber to achieve a total of 30 embryos. The B replicate of the negative control actually had 32 embryos and the C replicate of the 3.7 µg HBCD/L treatment group actually had 31 embryos.

REASON: Biologist oversight. It is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 1/25/01

LABORATORY MANAGEMENT — DATE 1/25/01

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WILDLIFE INTERNATIONAL LTD.

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/080100/RBT-ELS/SUB439 **DEVIATION NO.:** 2

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-112

DATE OF DEFACTO DEVIATION: September 21, 2000

DEVIATION: The protocol states that the temperature in each test chamber will be measured using a liquid-in-glass thermometer at the beginning of the test, at weekly intervals during the test, and at the end of the test. Temperature was not measured on Day 28 of the test.

REASON: Biologist oversight. Based on continuous temperature measurements recorded during the test, it is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 1/25/01

LABORATORY MANAGEMENT — DATE 1/25/01

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WILDLIFE INTERNATIONAL LTD.

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/080100/RBT-ELS/SUB439 **DEVIATION NO.:** 3

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-112

DATES OF DEFACTO DEVIATION: 8/29/00, 9/5/00, 9/19/00 and 10/3/00

DEVIATION: The protocol states that the temperature recorder measurements will be verified with a liquid-in-glass thermometer prior to test initiation, and verified/calibrated at weekly intervals thereafter. Recorder verification/calibration was not recorded for the above dates.

REASON: Biologist oversight. Based on weekly manual temperature measurements, it appears that the recorder calibration was correct. Consequently, it is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 1/25/01

LABORATORY MANAGEMENT — DATE 1/25/01

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WILDLIFE INTERNATIONAL LTD.

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/080100/RBT-ELS/SUB439 **DEVIATION NO.:** 4

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-112

DATE OF DEFACTO DEVIATION: September 5, 2000

DEVIATION: The protocol states that the rotameters and splits will be calibrated/checked prior to test initiation and at approximately weekly intervals thereafter. There was one 7-day interval during the test when rotameters and splits were not calibrated/checked.

REASON: Biologist oversight. The results of the test are based on measured concentrations. Consequently, it is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 1/25/01

LABORATORY MANAGEMENT — DATE 1/25/01

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WILDLIFE INTERNATIONAL LTD.

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/080100/RBT-ELS/SUB439 **DEVIATION NO.:** 5

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-112

DATE OF DEFACTO DEVIATION: November 7, 2000

DEVIATION: The protocol states that the fish will be fed three times daily on weekdays. On Day 75 of the test, the fish were only fed twice.

REASON: Biologist oversight. All fish were fed an equal amount of food. Consequently, it is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 2/9/01

LABORATORY MANAGEMENT — DATE 2/9/01

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PROJECT NO.: 439A-112
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WILDLIFE INTERNATIONAL LTD.

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): AN EARLY LIFE-STAGE TOXICITY TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/080100/RBT-ELS/SUB439 **DEVIATION NO.:** 6

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-112

DATE OF DEFACTO DEVIATION: November 14, 2000

DEVIATION: The protocol states that to ensure that the feeding rate per fish remains constant, rations will be adjusted on a weekly basis to account for losses due to mortality. The ration was not adjusted for the last 4 days of the test.

REASON: Biologist oversight. The fish were fed food left over from the previous week. It is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 2/12/01

LABORATORY MANAGEMENT — DATE 2/12/01

KRD:\439A112\prd6

Appendix 2

Test Substance Characterization

FINAL REPORT ON THE CHARACTERIZATION OF HEXABROMOCYCLODODECANE (HBCD) IN SUPPORT OF "HBCD FISH EARLY LIFE STAGE STUDY", CONDUCTED BY WILDLIFE INTERNATIONAL, LTD., EASTON, MD

Prepared for: Wendy K. Sherman, Study Monitor
American Chemistry Council
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Prepared by: Dr. Paul F. Ranken, Study Chemist
Research and Development Department
Albemarle Corporation
Albemarle Technical Center
8000 GSRI Avenue
Baton Rouge, LA 70820

ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT
FINAL REPORT ON THE CHARACTERIZATION OF HEXABROMOCYCLODODECANE (HBCD) IN SUPPORT OF "HBCD FISH EARLY LIFE STAGE STUDY", CONDUCTED BY WILDLIFE INTERNATIONAL, LTD., EASTON, MD

I.	Reference Protocol Number:	HBCD-08-08-2000
II.	Sponsor:	American Chemistry Council Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209 Study Monitor: Wendy K. Sherman
III.	Analytical Testing Facilities:	Albemarle Corporation Albemarle Technical Center 8000 GSRI Avenue Baton Rouge, LA 70820 Study Chemist: Paul F. Ranken, Ph. D.
IV.	Dates of Performance:	Study initiation date: August 8, 2000 Study completion date: December 7, 2000
V.	Test Article:	Hexabromocyclododecane (WIL Test substance No. 4615). The Test Article is a composite of commercial hexabromocyclododecane produced by Albemarle Corporation, Great Lakes Chemical Corporation and Eurobrom b.v. The composite was prepared by Wildlife International Ltd. for use in a "HBCD Fish Early Life Stage Study."
VI.	Objective/Methodology:	This study was initiated to characterize the Test Article which was to be used in a "HBCD Fish Early Life Stage Study" conducted by

Wildlife International.The identity of the Test Article was confirmed by Fourier Transform Infrared Spectroscopy using SOP No. ARS-284-R4. In this procedure, the sample infrared spectrum was compared to a standard refernce spectrum of HBCD. The refrence infrared spectrum was located in the Aldrich Condensed Phase High Resolution data library as 1-107A. The data library is an electronic collection of infrared spectra given in the Aldrich Library of FT-IR Spectra monographs. The Test Article was characterized by High Performance Liquid Chromatography using SOP No. ARS-432-R1. In this procedure, the presence of the three HBCD diastereomers (referred to as alpha, beta, and gamma isomers) was confirmed by comparing the retention times of the eluting peaks in the test sample to the typical retention times of the individual HBCD diastereomers. The distribution of the HBCD diastereomers in the Test Article is reported as area %. Chain of Custody and sample handling were conducted as per established standard operating procedures.

VII. Results:

The attached Conclusions and Test Article Analytical Data contains all of the test results from the study. The Test Article identity was confirmed by Fourier Transform Infrared Spectroscopy and further characterization was accomplished by HPLC. The distribution of the three HBCD diastereomers in the Test Article was 9.4 area% alpha, 6.3 area% beta and 84.3 area% gamma. There were no circumstances that may have affected the quality or integrity of the data.

VIII. Regulatory Requirements:

The study conformed to the requirements of EPA TSCA GLP's listed under 40 CFR Part 792 and the OECD [C(97)186/Final] Good Laboratory Practice Regulations.

IX. Data/Record Retention:

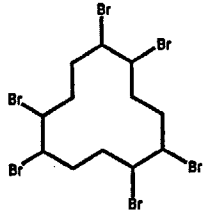
All original logbooks, spectra, original data and reports will be kept filed in the custody of the Study Chemist until the Toxicity study is completed, after which time they will be forwarded to the GLP Coordinator and stored in the designated Health and Environment archives at Albemarle Corporation, Health and Environment Department, 451 Florida Street, Baton Rouge, LA 70801.

Paul F. Ranken — December 7, 2000

Paul F. Ranken, Ph. D. STUDY CHEMIST — DATE

CONCLUSIONS AND TEST ARTICLE ANALYTICAL DATA

CHEMICAL NAME: Hexabromocyclododecane
C.A.S. No.: 3194-55-6
MOLECULAR FORMULA: $C_{12}H_{18}Br_6$
PHYSICAL FORM: White Powder
CHEMICAL STRUCTURE:



ANALYSIS	RESULTS			ANALYSIS DATES	ANALYST
FT-IR	The sample FT-IR spectrum matched that of the Aldrich reference spectrum. All spectra are on file with the original data.			August 11, 2000	W. T. Cobb
HPLC				August 10, 2000	J. S. Arroyave
	Typical Retention Time (minutes)	Retention Time; Analysis 1	Retention Time; Analysis 2		
Alpha isomer	13	13.6	13.6		
Beta isomer	15	15.3	15.3		
Gamma isomer	20	22.1	22.1		
		Area %; Analysis 1	Area %; Analysis 2	Area %; Average	
Alpha isomer		8.8	10	9.4	
Beta isomer		5.7	6.9	6.3	
Gamma isomer		85.5	83.1	84.3	
CONCLUSION: Based on these analytical data, the test article was identified and characterized as HBCD.					

Appendix 3

Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured During the 4-Week Period Immediately Preceding the Test

Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

	Mean	Range
Conductivity (µmhos/cm)	315 (N = 4)	310 – 320
Hardness (mg/L as $CaC0_3$)	131 (N = 4)	128 – 132
Alkalinity (mg/L as $CaC0_3$)	175 (N = 4)	172 – 178
pH	8.1 (N = 4)	7.9 - 8.1

Appendix 4
Analyses of Pesticides, Organics and Metals
In Wildlife International, Ltd. Well Water[1]

Component	Measured Concentration	Component	Measured Concentration
	Pesticides and Organics		
Aclonifen	<0.03 µg/L	Dichlorvos	<0.01 µg/L
Alachlor	<0.01 µg/L	Dicofol	<0.25 µg/L
Ametryn	<0.01 µg/L	Diethyltoluamide	<0.02 µg/L
Atrazine	<0.01 µg/L	Difenoconazole	<0.03 µg/L
Azinphos-ethyl	<0.04 µg/L	Dimethoate	<0.02 µg/L
Azinphos-methyl	<0.08 µg/L	Dimethomorph	<0.05 µg/L
Azoxystrobin	<0.25 µg/L	Disulfoton	<0.02 µg/L
Bifenthrin	<0.05 µg/L	DMST	<0.05 µg/L
Bioallethrin	<0.05 µg/L	Dodemorph	<0.01 µg/L
Bitertanol	<0.05 µg/L	Endosulfan-α	<0.01 µg/L
Bromacil	<0.05 µg/L	Endosulfan-β	<0.01 µg/L
Bromophos	<0.02 µg/L	Endosulfan-sulfte	<0.02 µg/L
Bromophos-ethyl	<0.02 µg/L	Epoxiconazole	<0.05 µg/L
Bromopropylate	<0.02 µg/L	Eptam	<0.02 µg/L
Bupirimate	<0.05 µg/L	Esfenvalerate	<0.02 µg/L
Carbaryl	<0.05 µg/L	Ethion	<0.05 µg/L
Carbofuran	<0.03 µg/L	Ethofumesate	<0.02 µg/L
Carboxin	<0.02 µg/L	Ethoprophos	<0.01 µg/L
Chlorfenvinphos	<0.02 µg/L	Etridiazole	<0.02 µg/L
Chloridazon	<0.05 µg/L	Etrimfos	<0.05 µg/L
Chlorpropham	<0.02 µg/L	Fenarimol	<0.05 µg/L
Chlorpyrifos	<0.01 µg/L	Fenchlorphos	<0.01 µg/L
Chlorpyrifos-methyl	<0.01 µg/L	Fenitrothion	<0.03 µg/L
Chlorothalonil	<0.04 µg/L	Fenoxycarb	<0.03 µg/L
Coumaphos	<0.02 µg/L	Fenpiclonil	<0.05 µg/L
Cyanazine	<0.05 µg/L	Fenpropathrin	<0.25 µg/L
Cyfluthrin	<0.05 µg/L	Fenpropimorph	<0.01 µg/L
Cypermethrin	<0.25 µg/L	Fenthion	<0.01 µg/L
Cyproconazole	<0.05 µg/L	Fenvalerate	<0.02 µg/L
Deltamethrin	<0.02 µg/L	Fluazifop-butyl	<0.02 µg/L
Demeton	<0.02 µg/L	Fluoroglycofen-ethyl	<0.02 µg/L
Demeton-O	<0.02 µg/L	Fluroxypyr-meptyl	<0.05 µg/L
Desethylatrazine	<0.01 µg/L	Flutolanil	<0.02 µg/L
Desisopropylatrazine	<0.02 µg/L	Fonophos	<0.01 µg/L
Desmetryn	<0.01 µg/L	Furalaxyl	<0.02 µg/L
Diazinon	<0.01 µg/L	Heptenophos	<0.02 µg/L
Dichlobenil	<0.01 µg/L	Imazalil	<0.01 µg/L
Dichloran	<0.03 µg/L	Iprodion	<0.05 µg/L
Dichlorbenzamide	<0.02 µg/L	Kresoxim-methyl	<0.02 µg/L
Dichlorfenthion	<0.01 µg/L	Lenacil	<0.05 µg/L
Dichlorfluanid	<0.03 µg/L	Lindane	<0.02 µg/L

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on October 14 and 15, 1999.

Appendix 4 (Continued)
Analyses of Pesticides, Organics and Metals
in Wildlife International, Ltd. Well Water[1]

Pesticides And Organics (Page 2)

Component	Measured Concentration	Component	Measured Concentration
Malathion	<0.02 µg/L	Methoxychlor	<0.01 µg/L
Metalaxyl	<0.05 µg/L	Metolachlor	<0.01 µg/L
Metamitron	<0.05 µg/L	Metribuzin	<0.02 µg/L
Metazachlor	<0.02 µg/L	Mevinphos	<0.01 µg/L
Methidathion	<0.02 µg/L	Nitrothal-Isopropyl	<0.05 µg/L
Paclobutazole	<0.05 µg/L	Pyrifenox-1	<0.01 µg/L
Parathion	<0.01 µg/L	Pyrifenox-2	<0.01 µg/L
Parathion-methyl	<0.01 µg/L	Pyrimethanil	<0.01 µg/L
Penconazole	<0.05 µg/L	Quizalofop-ethyl	<0.02 µg/L
Pendimethalin	<0.03 µg/L	Simazine	<0.01 µg/L
Permethrin-cis	<0.01 µg/L	Sulfotep	<0.02 µg/L
Permethrin-trans	<0.01 µg/L	Tebuconazole	<0.05 µg/L
Phosalone	<0.05 µg/L	Tebufenpyrad	<0.05 µg/L
Phosmet	<0.02 µg/L	Terbutryn	<0.01 µg/L
Phosphamidon-cis	<0.05 µg/L	Terbuthylazine	<0.01 µg/L
Pirimicarb	<0.01 µg/L	Tetrachlorvinphos	<0.01 µg/L
Pirimiphos-ethyl	<0.01 µg/L	Tetrahydroftalimide	<0.05 µg/L
Pirimiphos-methyl	<0.01 µg/L	Tetramethrin	<0.01 µg/L
Prochloraz	<0.02 µg/L	Thiabendazole	<0.05 µg/L
Procymidon	<0.01 µg/L	Thiometon	<0.04 µg/L
Prometryn	<0.01 µg/L	Tolclofos-methyl	<0.01 µg/L
Propachlor	<0.01 µg/L	Tolylfluanid	<0.04 µg/L
Propazine	<0.01 µg/L	Triadimefon	<0.05 µg/L
Propham	<0.02 µg/L	Triadimenol	<0.05 µg/L
Propiconazole	<0.05 µg/L	Triallate	<0.02 µg/L
Propoxur	<0.03 µg/L	Triazophos	<0.02 µg/L
Propyzamide	<0.02 µg/L	Trifluralin	<0.02 µg/L
Prosulfocarb	<0.02 µg/L	Vamidothion	<0.01 µg/L
Pyrazophos	<0.03 µg/L	Vinclozolin	<0.01 µg/L

Metals

Magnesium	11.0 mg/L	Nickel	<1.1 µg/L
Sodium	18.0 mg/L	Copper	<0.7 µg/L
Calcium	29 mg/L	Zinc	<0.25 µg/L
Iron	<0.015 mg/L	Molybdenum	<0.3 µg/L
Potassium	1.1 mg/L	Silver	<0.2 µg/L
Aluminum	<0.02 mg/L	Cadmium	<0.1 µg/L
Manganese	<0.1 µg/L	Arsenic	<0.5 µg/L
Beryllium	<0.2 µg/L	Mercury	<0.025 µg/L
Chromium	<0.5 µg/L	Selenium	<0.5 µg/L
Cobalt	<0.2 µg/L		

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on October 14 and 15, 1999.

Appendix 5

THE ANALYSIS OF HEXABROMOCYCLODODECANE (HBCD-AS SEPARATE APLHA, BETA, AND GAMMA DIASTEREOMERS) CONCENTRATIONS IN FRESHWATER
IN SUPPORT OF
WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439A-112

REPORT APPROVAL

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

TITLE: Hexabromocyclododecane (HBCD): An Early Life-Stage Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439A-112

PRINCIPAL INVESTIGATOR:

Jon A. MacGregor, B.S.
Scientist

7/12/01
DATE

MANAGEMENT:

Willard B. Nixon, Ph.D.
Director, Analytical Chemistry

7/12/01
DATE

Introduction

Freshwater samples were collected from a flow-through aquatic test to determine the effect of hexabromocyclododecane (HBCD) on the early life-stage of the rainbow trout (*Oncorhynchus mykiss*). The study was conducted by Wildlife International, Ltd. and identified as Project Number 439A-112. The analyses of freshwater samples were performed at Wildlife International, Ltd. by high performance liquid chromatography with mass spectrometry (HPLC/MS). Water samples were collected between August 23 and November 20, 2000. All sample processing and analyses were initiated upon each day of collection.

Analytical Standards

Separate analytical standards of the alpha, beta and gamma diastereomers of HBCD were received from Albermarle Chemical Corporation on March 15, 2000 and assigned the Wildlife International, Ltd identification numbers of 5204A, 5204B, and 5204C, respectively, upon receipt. The standards were described as a white powders and were identified as: SAYTEX HBCD-LM (Alpha); SAYTEX HBCD-LM (Beta); SAYTEX HBCD-LM (Gamma); CAS number 3194-55-6. The standards had a reported purities of 98% and were stored under ambient conditions. These analytical standards were used to prepare combined calibration standards and matrix fortification standards for this study. The prominent gamma diastereomer was used as a marker for quantitation of the HBCD formulated test substance used during the definitive study exposure.

Analytical Method

The analytical methodology for the analysis of HBCD in freshwater samples is described below.

Freshwater samples (50-mL aliquots) were volumetrically collected and transferred to 125-mL separatory funnels that contained 25 mL of dichloromethane (DCM). The separatory funnels were shaken for approximately one minute. After the aqueous and organic phases separated, the organic phase (lower layer) of each sample was drained into a 125-mL round-bottom flask. The extraction procedure was repeated with a second 25-mL aliquot of DCM. The organic extracts from the second extraction were combined with the extracts from the first extraction in the appropriate flasks. The extracts were rotary evaporated under vacuum in water baths maintained at approximately 40°C until

1-2 mL of each extract remained. The remaining DCM was evaporated to dryness under a gentle stream of nitrogen and the residues were reconstituted with the requisite volume of 75% ACN: 25% H_2O. Aliquots of the reconstituted extracts were transferred to autosampler vials and submitted for analysis. A method flow chart for the analysis of HBCD in freshwater is presented in Figure 1.

Freshwater quality control (QC) samples (matrix blanks and fortifications) were processed in the same manner as the test samples, except each QC sample was prepared by first adding 50 mL of freshwater to a 125-mL separatory funnel, fortifying the water with the appropriate HBCD stock solution using a gas-tight syringe (for matrix fortification samples) and then adding the initial 25-mL aliquot of DCM. Matrix blank samples were not fortified with the test substance.

Concentrations of HBCD were determined by high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) equipped with a Perkin-Elmer API 100LC Mass Spectrometer and APCI Heated Nebulizer Source. Chromatographic separations were achieved using a YMC AM C-18 analytical column (150 mm × 4.6 mm, 3-µm particle size). The typical instrument parameters are summarized in Table 1.

Fortification/Calibration Stocks and Standards

Three separate primary stocks of alpha, beta, and gamma HBCD diastereomers were prepared for use in the study by accurately weighing appropriate amounts of the individual analytical standards and volumetrically dissolving each in tetrahydrofuran (THF) solvent. The individual primary stock solutions contained 100 µg a.i. (alpha, beta, or gamma HBCD)/mL. Combined working standard solutions were prepared from these primary stock solutions by dilution as appropriate with THF, yielding standard solutions that ranged from 0.100 to 10.0 µg alpha, beta, gamma HBCD/mL. The combined working standards were used to prepare both the concurrent matrix fortification samples and calibration standards using the following dilution schemes:

Combined Working Standard Solutions:

Stock Concentration (µg a.i./mL)	Stock Aliquot (mL)	Final Diluted Volume (mL)	Combined Standard Concentration (µg a.i./L)	Dilution Solvent
100(alpha)	5.00	50.0	10.0	THF
100(beta)	5.00			
100(gamma)	5.00			
10.0(combined)	5.00	50.0	1.00	THF
1.00(combined)	5.00	50.0	0.100	THF

Combined Calibration Standards:

Combined Stock Concentration (µg a.i./mL)	Stock Aliquot (mL)	Final Diluted Volume (mL)	Combined Standard Concentration (µg a.i./L)	Dilution Solvent
1.00	0.100	100	1.00	75% ACN: 25% H_2O
1.00	0.250	100	2.50	75% ACN: 25% H_2O
1.00	0.500	100	5.00	75% ACN: 25% H_2O
1.00	0.750	100	7.50	75% ACN: 25% H_2O
1.00	1.00	100	10.0	75% ACN: 25% H_2O

All stocks and standards were prepared using a combination of Class A volumetric flasks, volumetric pipets, and/or gas tight syringes. Each stock solution was assigned a unique identification code, which was recorded on a stock preparation log sheet.

Calibration Curve and Limit of Quantitation

Combined calibration standards of alpha, beta, and gamma HBCD diastereomers ranging in concentration from 1.00 to 10.0 µg a.i./L, were analyzed with each freshwater sample set. For each analysis, a set of five calibration standards was injected at the beginning and end of the analytical run. In addition, a minimum of one standard was injected following every five test samples. A calibration curve for each diastereomer was derived from a weighted (1/x) regression analysis using the instrumental responses of each individual component of the combined calibration standards. Separate regression equations for the potential quantitation of each diastereomer were generated using the peak

area responses of each individual component versus their respective concentrations in the combined standards. Typical calibration curves for the alpha, beta, and gamma HBCD diasteriomers are presented in Figures 2, 3, and 4, respectively.

For quantitation of the HCBD test substance in the definitive study samples, the prominent gamma component (based on relative area % in the formulated test substance) was selected as the marker. All sample processing and dilutions were performed for quantitation using the gamma component. This typically resulted in alpha and beta results at or below their limits of quantitation, calculated based on the dilution factors for the quantitation of the gamma component. The alpha and beta diastereomers were monitored to observe the potential for any significant changes or shifts in the relative distributions of the HBCD diastereomers in the aquatic test system during the definitive study. The concentration of HBCD formulation in the freshwater samples was determined by substituting the peak area responses of each HBCD component into the appropriate weighted (1/x) regression equation as follows:

$$\text{HBCD in sample } (\mu g/L) = [(\text{peak area} - \text{y-intercept})/\text{slope}/\text{purity}] * \text{dilution factor}$$

$$\% \text{ Recovery} = \frac{\text{measured HBCD concentration } (\mu g/L)}{\text{nominal HBCD concentration } (\mu g/L)} \times 100$$

Representative ion chromatograms of low and high combined calibration standards are presented in Figures 5 and 6, respectively.

The method limit of quantitation (LOQ) for freshwater control samples was 0.0400 µg alpha, beta, gamma HBCD /L, calculated as the product of the lowest calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blank samples (0.0400) analyzed concurrently with the test samples. The LOQ for the alpha and beta diastereomers for freshwater treatment levels increased with sample nominal concentrations as a result of sample processing and dilutions for the quantitation of the test substance using the prominent HBCD gamma diastereomer.

Freshwater Matrix Blank and Fortification Samples

Along with the actual freshwater sample analyses, 14 freshwater matrix blank samples were analyzed to determine possible interferences. No interferences were observed at or above the LOQ

during the test study (Table 2). A representative ion chromatogram of a freshwater matrix blank sample is presented in Figure 7.

Freshwater samples were fortified with alpha, beta, and gamma HBCD at 0.100, 1.00 and 10.0 µg a.i./L using a combined standard solution of HBCD prepared in THF and analyzed concurrently with each sample set to determine the procedural recovery. The procedural recoveries of alpha, beta and gamma HBCD for the study ranged from 91.6 to 114%, 92.9 to 112%, and 95.4 to 125% of nominal concentrations, respectively (Table 2). The overall mean procedural recovery for alpha, beta, and gamma for the study were 102 ± 5.4 %, 101 ± 4.6 %, and 102 ± 5.1 %, respectively. A representative ion chromatogram of a freshwater matrix fortification is presented in Figure 8.

Example Calculations

The analytical result and percent recovery for freshwater sample 439A-112-3, from the 0.43 µg/L nominal HBCD treatment group, was calculated based on the gamma diastereomer of HBCD using the following equations:

$$\text{HBCD in sample (µg/L)} = \frac{\text{(peak area – y-intercept)}}{\text{slope x purity}} \times \text{dilution factor}$$

Peak area = 5388
Y-intercept = -22.82420
Slope = 703.12183
(Note: Regression = 1/x weighted)
Initial volume (V_i) = 50.0 mL
Final volume (V_f) = 2.00 mL
Dilution factor (V_f/V_i) = 0.0400
Purity (gamma) = 84.3%

$$\text{HBCD in sample (µg/L)} = \frac{(5388 - -22.82420)}{703.12183 \times 0.843} \times 0.0400$$

$$\text{HBCD in sample (µg/L)} = 0.365 \text{ µg/L}$$

$$\text{\% Recovery in sample} = \frac{\text{measured HBCD concentration (µg/L)}}{\text{nominal HBCD concentration (µg/L)}} \times 100$$

$$\text{\% Recovery in sample} = \frac{0.365 \text{ µg/L}}{0.43 \text{ µg/L}} \times 100$$

$$\text{\% Recovery in sample} = 85.0 \text{ \%}$$

RESULTS

Freshwater Sample Analysis

Freshwater samples were collected and analyzed for HBCD concentrations on August 23, 2000 (pre-test Day -1), at study initiation (Day 0) on August 24, 2000, on Days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and on Day 88 (test termination) on November 21, 2000.

Measured concentrations of HBCD formulated test substance in the pre-test diluter verification treatment samples ranged from 51.6 to 89.7 % of nominal concentrations (Table 3). Measured concentrations of HBCD formulated test substance in the samples collected at study initiation (Day 0) ranged from 46.4 to 85.0 % of nominal concentrations (Table 4). Measured concentrations of HBCD formulated test substance in samples collected on Days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 88 (test termination) of the study ranged from 58.4 to 81.7 %, 56.7 to 83.0 %, 43.6 to 70.0 %, 37.5 to 49.5 %, 48.0 to 69.2 %, 51.0 to 66.2 %, 54.6 to 66.8 %, 40.1 to 76.4 %, 45.4 to 59.3 %, 40.1 to 50.2 %, 41.5 to 57.2 %, 32.7 to 60.1 %, and 28.5 to 53.5 %, respectively. Representative ion chromatograms of freshwater samples on Day 0 (initiation) and Day 88 (termination) are presented in Figure 9 and 10, respectively.

The analytical method was designed to be able to monitor the aqueous samples for the separate detection of the alpha, beta, and gamma HBCD diastereomers. While trace residues of the alpha and beta diastereomers were evident in the samples, they were below the established limits of quantitation. By comparison of the resulting chromatograms from study initiation through study termination, it can be concluded that the relative distribution of the HBCD diastereomers remained constant during the definitive study. Additionally, the gamma diastereomer measured results for the study were consistent, further indicating HBCD diastereomer distribution stability in the test system.

Table 1

Typical HPLC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 100LC Mass Spectrometer
SOURCE:	Perkin-Elmer Heated Nebulizer Operated in Selective Ion Monitoring (SIM) Mode
ANALYTICAL COLUMN:	YMC AM C-18 (150 mm × 4.6 mm, 3-µm particle size)
OVEN TEMPERATURE:	40°C
STOP TIME:	12.00 min
FLOW RATE:	0.750 mL/min
MOBILE PHASE:	85% Acetonitrile :15% NANOpure Water® with 0.1% Formic Acid:
INJECTION VOLUME:	100 µL
HBCD DIASTEREOMER PEAK RETENTION TIMES:	Alpha- ~ 6.41 minutes Beta- ~ 7.01 minutes Gamma- ~ 9.01 minutes
HBCD MONITORED MASS:	640.7 amu

Table 2

Matrix Blanks and Fortifications Analyzed Concurrently During Freshwater Sample Analyses

Sample Number (439A-112-)	Concentration of HBCD (µg a.i./L)				Percent Recovery[2]		
	Fortified	Measured (alpha[1])	Measured (beta)	Measured (gamma)	alpha	beta	gamma
PT-MAB-1	0.00	<0.0400	<0.0400	<0.0400	--	--	--
PT-MAS-1	0.100	0.108	0.107	0.104	108	107	104
PT-MAS-2	1.00	1.09	1.10	1.05	109	110	105
PT-MAS-3	10.0	10.9	11.2	11.0	109	112	110
MAB-1	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-1	0.100	0.106	0.0980	0.104	106	98.0	104
MAS-2	1.00	0.999	1.00	1.10	99.9	100	110
MAS-3	10.0	9.76	9.86	10.4	97.6	98.6	104
MAB-2	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-4	0.100	0.107	0.109	0.108	107	109	108
MAS-5	1.00	0.988	0.974	0.992	98.8	97.4	99.2
MAS-6	10.0	10.1	9.82	10.4	101	98.2	104
MAB-3	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-7	0.100	0.0962	0.102	0.0978	96.2	102	97.8
MAS-8	1.00	0.979	0.975	1.00	97.9	97.5	100
MAS-9	10.0	9.81	10.0	9.94	98.1	100	99.4
MAB-4	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-10	0.100	0.102	0.109	0.102	102	109	102
MAS-11	1.00	0.964	0.995	0.954	96.4	99.5	95.4
MAS-12	10.0	9.24	9.43	9.68	92.4	94.3	96.8
MAB-5	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-13	0.100	0.104	0.100	0.101	104	100	101
MAS-14	1.00	1.07	1.01	0.995	107	101	99.5
MAS-15	10.0	10.0	10.2	9.75	100	102	97.5
MAB-6	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-16	0.100	0.101	0.0967	0.0981	101	96.7	98.1
MAS-17	1.00	1.03	1.04	1.02	103	104	102
MAS-18	10.0	10.0	9.30	9.93	100	93.0	99.3
MAB-7	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-19	0.100	0.0944	0.105	0.100	94.4	105	100
MAS-20	1.00	1.06	1.02	1.00	106	102	100
MAS-21	10.0	9.73	9.29	9.67	97.3	92.9	96.7

[1] The limit of quantitation (LOQ) was 0.0400 µg a.i./L, calculated as the product of the lowest combined calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blank sample (0.0400).

[2] Results were generated using MacQuan, version 1.5 and 1.6 software. Manual calculations may differ slightly.

Table 2 (Continued)

Matrix Blanks and Fortifications Analyzed Concurrently During Freshwater Sample Analyses

Sample Number (439A-112-)	Concentration of HBCD (µg a.i./L)				Percent Recovery[2]		
	Fortified	Measured (alpha[1])	Measured (beta)	Measured (gamma)	alpha	beta	gamma
MAB-8	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-22	0.100	0.0960	0.0996	0.0990	96.0	99.6	99.0
MAS-23	1.00	0.977	0.977	1.04	97.7	97.7	104
MAS-24	10.0	9.16	10.1	9.73	91.6	101	97.3
MAB-9	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-25	0.100	0.0994	0.0957	0.104	99.4	95.7	104
MAS-26	1.00	1.02	1.02	1.00	102	102	100
MAS-27	10.0	9.86	9.49	9.90	98.6	94.9	99.0
MAB-10	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-28	0.100	0.103	0.0998	0.0980	103	99.8	98.0
MAS-29	1.00	1.04	1.01	1.05	104	101	105
MAS-30	10.0	9.24	9.57	9.81	92.4	95.7	98.1
MAB-11	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-31	0.100	0.106	0.0973	0.100	106	97.3	100
MAS-32	1.00	0.982	1.02	0.972	98.2	102	97.2
MAS-33	10.0	9.87	9.94	9.63	98.7	99.4	96.3
MAB-12	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-34	0.100	0.114	0.107	0.101	114	107	101
MAS-35	1.00	1.04	1.07	1.01	104	107	101
MAS-36	10.0	10.7	10.5	10.6	107	105	106
MAB-13	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-37	0.100	0.100	0.0937	0.106	100	93.7	106
MAS-38	1.00	0.965	0.973	1.07	96.5	97.3	107
MAS-39	10.0	10.7	9.83	10.3	107	98.3	103
MAB-14	0.00	<0.0400	<0.0400	<0.0400	--	--	--
MAS-40	0.100	0.114	0.105	0.125	114	105	125
MAS-41	1.00	1.06	1.00	1.08	106	100	108
MAS-42	10.0	10.5	10.0	10.2	105	100	102
				Mean =	102%	101%	102%
				Std. Dev. =	5.3%	4.6%	5.1%
				N =	45	45	45

[1]The limit of quantitation (LOQ) was 0.0400 µg a.i./L, calculated as the product of the lowest combined calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blank sample (0.0400).
[2]Results were generated using MacQuan, version 1.5 and 1.6 software. Manual calculations may differ slightly.

Table 3

Measured Concentrations of Hexabromocyclododecane (HBCD) in Pre-Test Diluter Verification Samples

Nominal Test Concentration (µg/L)	Sample Number (439A-112-)	Sampling Time (Day)	Measured Concentration of HBCD (µg a.i./L)[1]			Corrected HBCD Concentration (µg/L)	Percent of Nominal[2]
			Alpha	Beta	gamma		
0.0 (Negative Control)	PT-1	-1	<0.0400	<0.0400	<0.0400	--	--
0.0 (Solvent Control)	PT-2	-1	<0.0400	<0.0400	<0.0400	--	--
0.43	PT-3	-1	<0.0400	<0.0400	0.325	0.386	89.7
0.85	PT-4	-1	<0.100	<0.100	0.586	0.695	81.8
1.7	PT-5	-1	<0.200	<0.200	0.993	1.18	69.3
3.4	PT-6	-1	<0.400	<0.400	1.58	1.87	55.1
6.8	PT-7	-1	<0.800	<0.800	2.96	3.51	51.6

[1] The limit of quantitation (LOQ) was 0.0400 µg a.i./L, calculated as the product of the lowest calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blank sample (0.0400). The LOQ for the alpha and beta diastereomers treatment levels increased with sample concentration as a result sample processing/dilution for quantitation of the test substance using the prominent HBCD gamma diastereomer.

[2] Results were generated using MacQuan, version 1.5 software. Manual calculations may differ slightly.

Table 4

Measured Concentrations of Hexabromocyclododecane (HBCD) in Freshwater Samples from a Rainbow Trout Bioconcentration Test

Nominal Test Concentration (µg/L)	Sample Number (439A-112-)	Sampling Time (Day)	Measured Concentration of HBCD[1] (µg a.i./L)			Corrected HBCD Test Substance Concentration (µg/L)	Percent of Nominal[2]
			alpha	beta	gamma		
0.0 (Negative Control)	1	0	<0.0400	<0.0400	<0.0400	–	–
	8	7	<0.0400	<0.0400	<0.0400	–	–
	15	14	<0.0400	<0.0400	<0.0400	–	–
	22	21	<0.0400	<0.0400	<0.0400	–	–
	29	28	<0.0400	<0.0400	<0.0400	–	–
	36	35	<0.0400	<0.0400	<0.0400	–	–
	43	42	<0.0400	<0.0400	<0.0400	–	–
	50	49	<0.0400	<0.0400	<0.0400	–	–
	57	56	<0.0400	<0.0400	<0.0400	–	–
	64	63	<0.0400	<0.0400	<0.0400	–	–
	71	70	<0.0400	<0.0400	<0.0400	–	–
	78	77	<0.0400	<0.0400	<0.0400	–	–
	85	84	<0.0400	<0.0400	0.0410	0.0486[3]	–
	92	88	<0.0400	<0.0400	<0.0400	–	–
0.0 (Solvent Control)	2	0	<0.0400	<0.0400	<0.0400	–	–
	9	7	<0.0400	<0.0400	<0.0400	–	–
	16	14	<0.0400	<0.0400	<0.0400	–	–
	23	21	<0.0400	<0.0400	<0.0400	–	–
	30	28	<0.0400	<0.0400	<0.0400	–	–
	37	35	<0.0400	<0.0400	<0.0400	–	–
	44	42	<0.0400	<0.0400	<0.0400	–	–
	51	49	<0.0400	<0.0400	<0.0400	–	–
	58	56	<0.0400	<0.0400	<0.0400	–	–
	65	63	<0.0400	<0.0400	<0.0400	–	–
	72	70	<0.0400	<0.0400	<0.0400	–	–
	79	77	<0.0400	<0.0400	<0.0400	–	–
	86	84	<0.0400	<0.0400	<0.0400	–	–
	93	88	<0.0400	<0.0400	<0.0400	–	–

[1] The limit of quantitation (LOQ) was 0.0400 µg a.i./L, calculated as the product of the lowest calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blank sample (0.0400). The LOQ for the alpha and beta diastereomers treatment levels increased with sample concentration as a result sample processing/dilution for quantitation of the test substance using the prominent HBCD gamma diastereomer.

[2] Results were generated using MacQuan, version 1.5 software. Manual calculations may differ slightly.

[3] Contribution of gamma HBCD from extraction solvent (dichloromethane).

Table 4 (Continued)

Measured Concentrations of Hexabromocyclododecane (HBCD) in Freshwater Samples from a Rainbow Trout Bioconcentration Test

Nominal Test Concentration (µg/L)	Sample Number (439A-112-)	Sampling Time (Day)	Measured Concentration of HBCD[1] (µg a.i./L)			Corrected HBCD Test Substance Concentration (µg/L)	Percent of Nominal[2]
			alpha	beta	gamma		
0.43	3	0	<0.0400	<0.0400	0.308	0.365	85.0
	10	7	<0.0400	<0.0400	0.296	0.351	81.7
	17	14	<0.0400	<0.0400	0.252	0.299	69.5
	24	21	<0.0400	<0.0400	0.173	0.205	47.7
	31	28	<0.0400	<0.0400	0.136	0.161	37.5
	38	35	<0.0400	<0.0400	0.251	0.298	69.2
	45	42	<0.0400	<0.0400	0.185	0.219	51.0
	52	49	<0.0400	<0.0400	0.236	0.280	65.1
	59	56	<0.0400	<0.0400	0.228	0.270	62.9
	66	63	<0.0400	<0.0400	0.190	0.225	52.4
	73	70	<0.0400	<0.0400	0.156	0.185	43.0
	80	77	<0.0400	<0.0400	0.159	0.189	43.9
	87	84	<0.0400	<0.0400	0.218	0.259	60.1
	94	88	<0.0400	<0.0400	0.194	0.230	53.5
0.85	4	0	<0.100	<0.100	0.406	0.482	56.7
	11	7	<0.100	<0.100	0.577	0.684	80.5
	18	14	<0.100	<0.100	0.545	0.647	76.1
	25	21	<0.100	<0.100	0.379	0.450	52.9
	32	28	<0.100	<0.100	0.345	0.409	48.1
	39	35	<0.100	<0.100	0.360	0.427	50.2
	46	42	<0.100	<0.100	0.474	0.562	66.2
	53	49	<0.100	<0.100	0.417	0.495	58.2
	60	56	<0.100	<0.100	0.287	0.340	40.1
	67	63	<0.100	<0.100	0.353	0.419	49.3
	74	70	<0.100	<0.100	0.328	0.389	45.8
	81	77	<0.100	<0.100	0.410	0.486	57.2
	88	84	<0.100	<0.100	0.311	0.369	43.4
	95	88	<0.100	<0.100	0.337	0.400	47.0

[1] The limit of quantitation (LOQ) was 0.0400 µg a.i./L, calculated as the product of the lowest calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blank sample (0.0400). The LOQ for the alpha and beta diastereomers treatment levels increased with sample concentration as a result sample processing/dilution for quantitation of the test substance using the prominent HBCD gamma diastereomer.

[2] Results were generated using MacQuan, version 1.5 software. Manual calculations may differ slightly.

Table 4 (Continued)

Measured Concentrations of Hexabromocyclododecane (HBCD) in Freshwater Samples from a Rainbow Trout Bioconcentration Test

Nominal Test Concentration (µg/L)	Sample Number (439A-112-)	Sampling Time (Day)	Measured Concentration of HBCD[1] (µg a.i./L)			Corrected HBCD Test Substance Concentration (µg/L)	Percent of Nominal[2]
			alpha	beta	gamma		
1.7	5	0	<0.200	<0.200	0.715	0.848	49.9
	12	7	<0.200	<0.200	0.870	1.03	60.7
	19	14	<0.200	<0.200	0.813	0.964	56.7
	26	21	<0.200	<0.200	0.731	0.867	51.0
	33	28	<0.200	<0.200	0.547	0.649	38.2
	40	35	<0.200	<0.200	0.688	0.816	48.0
	47	42	<0.200	<0.200	0.770	0.913	53.7
	54	49	<0.200	<0.200	0.783	0.929	54.6
	61	56	<0.200	<0.200	0.844	1.00	58.9
	68	63	<0.200	<0.200	0.850	1.01	59.3
	75	70	<0.200	<0.200	0.594	0.705	41.4
	82	77	<0.200	<0.200	0.607	0.720	42.4
	89	84	<0.200	<0.200	0.612	0.726	42.7
	96	88	<0.200	<0.200	0.409	0.485	28.5
3.4	6	0	<0.400	<0.400	1.33	1.58	46.4
	13	7	<0.400	<0.400	1.84	2.18	64.2
	20	14	<0.400	<0.400	2.38	2.82	83.0
	27	21	<0.400	<0.400	1.25	1.48	43.6
	34	28	<0.400	<0.400	1.08	1.28	37.7
	41	35	<0.400	<0.400	1.80	2.14	62.8
	48	42	<0.400	<0.400	1.66	1.97	57.9
	55	49	<0.400	<0.400	1.75	2.08	61.1
	62	56	<0.400	<0.400	2.19	2.60	76.4
	69	63	<0.400	<0.400	1.30	1.54	45.4
	76	70	<0.400	<0.400	1.15	1.36	40.1
	83	77	<0.400	<0.400	1.19	1.41	41.5
	90	84	<0.400	<0.400	0.937	1.11	32.7
	97	88	<0.400	<0.400	1.22	1.45	42.6

[1] The limit of quantitation (LOQ) was 0.0400 µg a.i./L, calculated as the product of the lowest calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blank sample (0.0400). The LOQ for the alpha and beta diastereomers treatment levels increased with sample concentration as a result sample processing/dilution for quantitation of the test substance using the prominent HBCD gamma diastereomer.

[2] Results were generated using MacQuan, version 1.5 software. Manual calculations may differ slightly.

Table 4 (Continued)

Measured Concentrations of Hexabromocyclododecane (HBCD) in Freshwater Samples from a Rainbow Trout Bioconcentration Test

Nominal Test Concentration (µg/L)	Sample Number (439A-112-)	Sampling Time (Day)	Measured Concentration of HBCD[1] (µg a.i./L)			Corrected HBCD Test Substance Concentration (µg/L)	Percent of Nominal[2]
			alpha	beta	gamma		
6.8	7	0	<0.800	<0.800	2.86	3.39	49.9
	14	7	<0.800	<0.800	3.35	3.97	58.4
	21	14	<0.800	<0.800	3.99	4.73	69.6
	28	21	<0.800	<0.800	4.02	4.77	70.1
	35	28	<0.800	<0.800	2.84	3.37	49.5
	42	35	<0.800	<0.800	3.26	3.87	56.9
	49	42	<0.800	<0.800	3.35	3.97	58.4
	56	49	<0.800	<0.800	3.83	4.54	66.8
	63	56	<0.800	<0.800	2.94	3.49	51.3
	70	63	<0.800	<0.800	2.98	3.54	52.0
	77	70	<0.800	<0.800	2.88	3.42	50.2
	84	77	<0.800	<0.800	2.85	3.38	49.7
	91	84	<0.800	<0.800	2.32	2.75	40.4
	98	88	<0.800	<0.800	2.45	2.91	42.7

[1] The limit of quantitation (LOQ) was 0.0400 µg a.i./L, calculated as the product of the lowest calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blank sample (0.0400). The LOQ for the alpha and beta diastereomers treatment levels increased with sample concentration as a result sample processing/dilution for quantitation of the test substance using the prominent HBCD gamma diastereomer.

[2] Results were generated using MacQuan, version 1.5 software. Manual calculations may differ slightly.

METHOD OUTLINE FOR THE ANALYSIS OF HEXABROMOCYCLODODECANE (HBCD) IN FRESHWATER

Prepare each quality control (QC) sample by adding 50 mL of freshwater to a 125-mL separatory funnel. Fortify each QC sample with the appropriate HBCD stock solution. The matrix blank sample will not be fortified. Add 25 mL of dichloromethane (DCM) to each separatory funnel.

↓

Transfer 50 mL of each study test solution directly into a separatory funnel containing 25 mL of DCM.

↓

Shake the QC and study sample solutions (with venting) for approximately one minute and allow the two phases to separate.

↓

For each sample, drain the organic (lower) phase into a 125-mL round-bottom flask. Add a second 25-mL aliquot of DCM to the aqueous phase remaining in the funnel and perform a second extraction.

↓

For each sample, combine the organic phase from the second extraction with the organic phase of the first extraction in the same round-bottom flask.

↓

Rotary evaporate the organic extracts to 1-2 mL under vacuum and a water bath set at approximately 40°C.

↓

Evaporate the residual DCM in each flask to dryness under a gentle stream of nitrogen. Reconstitute the residues with appropriate volume of 75% acetonitrile (ACN): 25% water dilution solvent.

↓

Transfer an aliquot of the diluted extracts into an autosampler vial and ampulate. Submit for LC/MS analysis.

Figure 1. Method flow chart for the analysis of hexabromocyclododecane (HBCD) in freshwater.

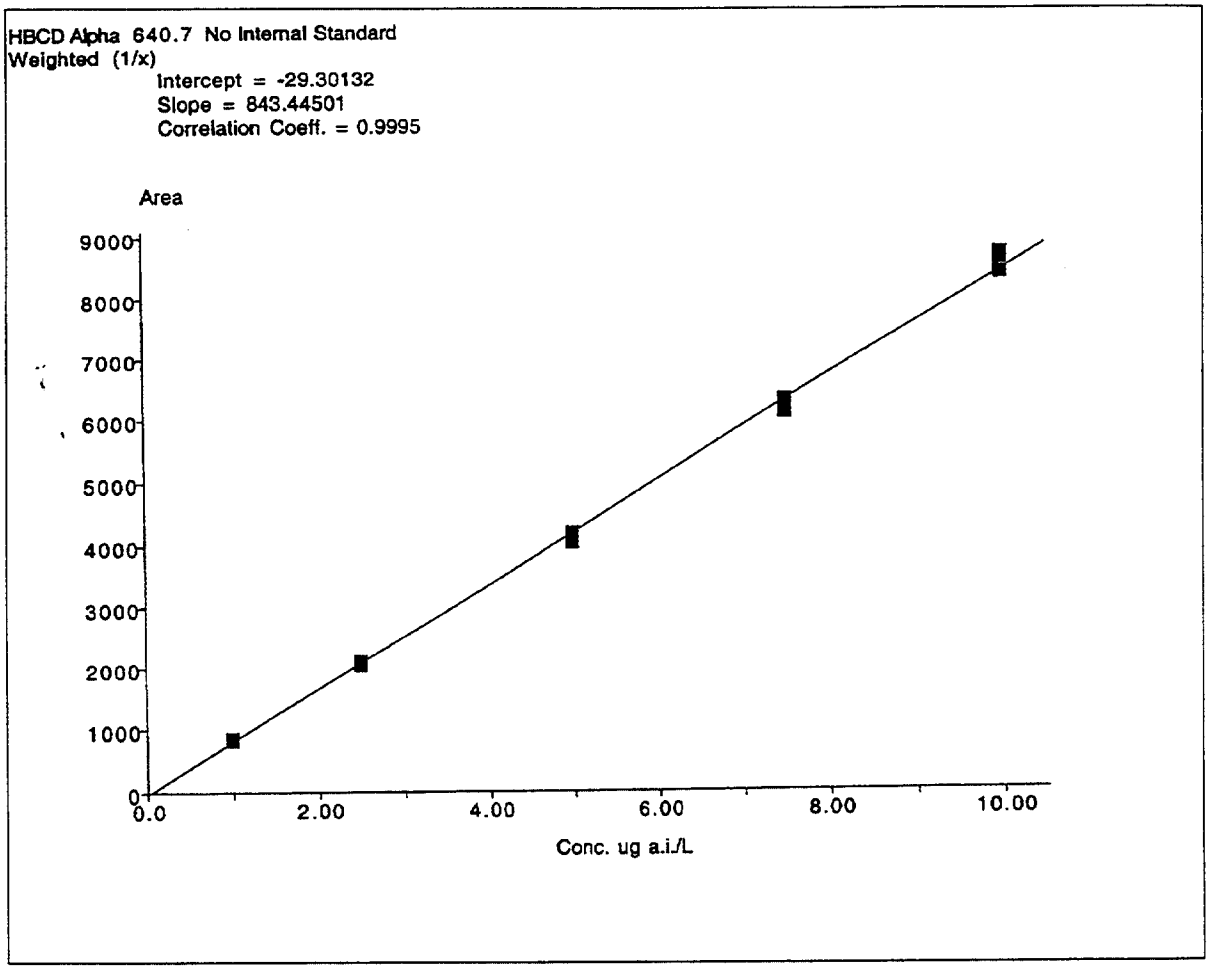


Figure 2. A typical calibration curve for alpha diastereomer of hexabromocyclododecane (HBCD). Slope = 2078.28491; Intercept = 268.05856; r = 0.9993. Curve is weighted (1/x).

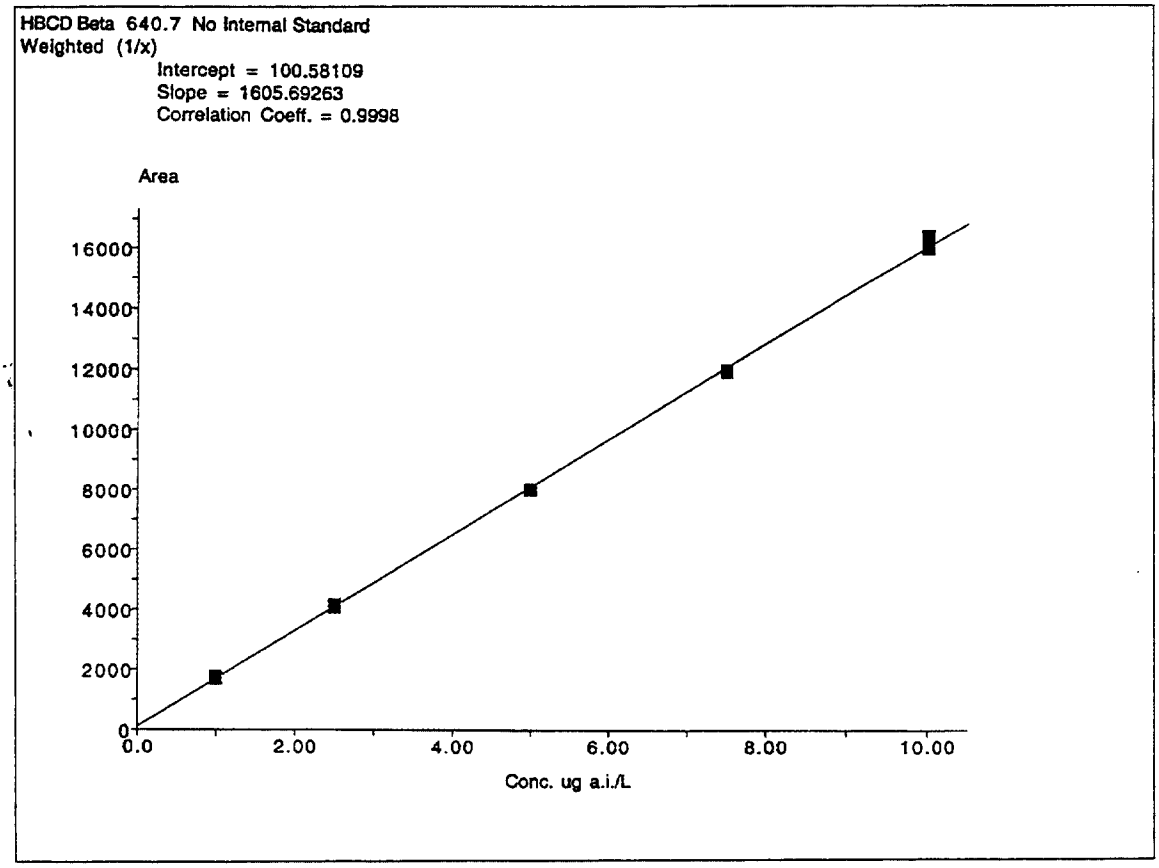


Figure 3. A typical calibration curve for beta diastereomer of hexabromocyclododecane (HBCD). Slope = 2078.28491; Intercept = 268.05856; r = 0.9993. Curve is weighted (1/x).

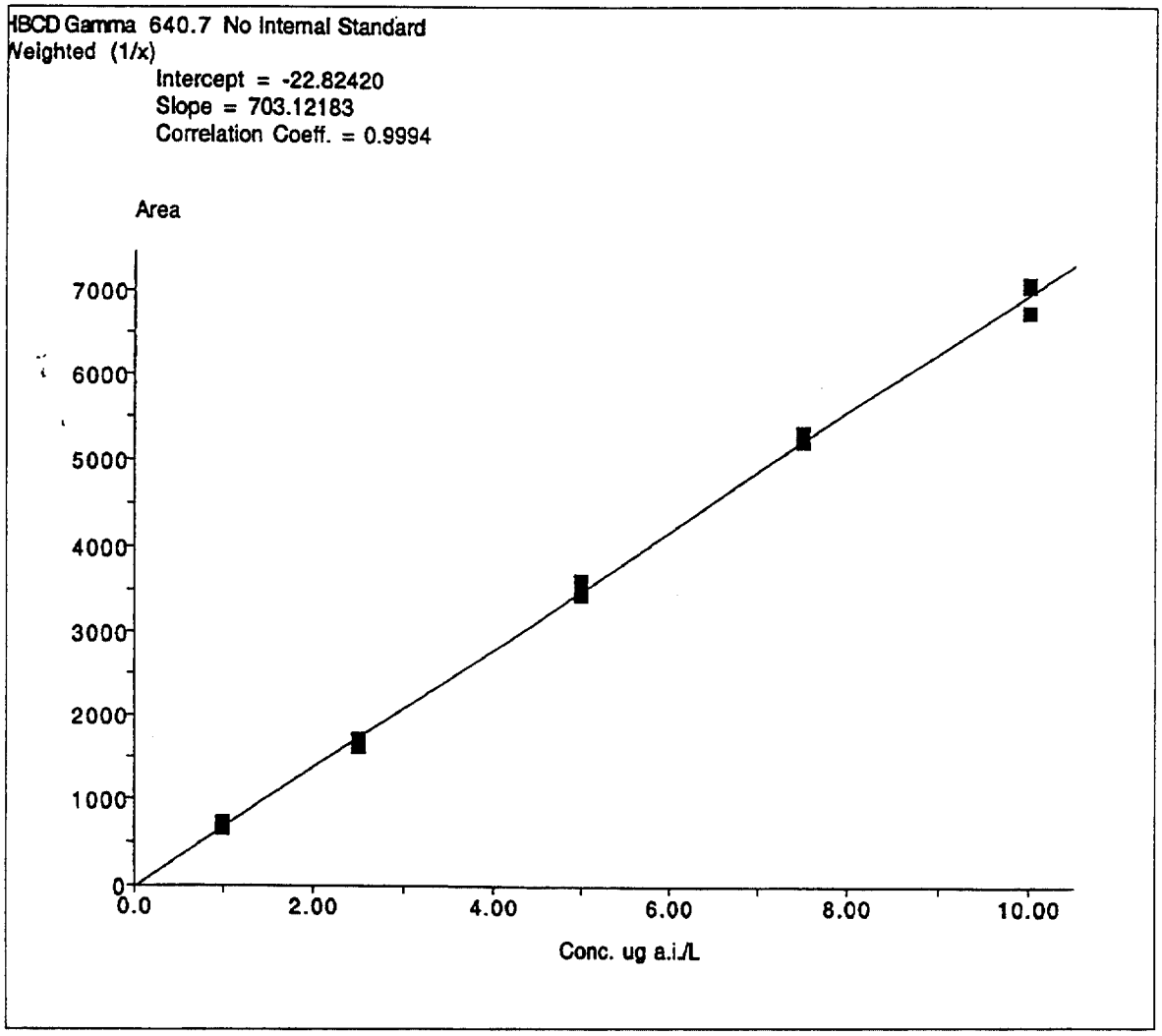


Figure 4. A typical calibration curve for gamma diastereomer of hexabromocyclododecane (HBCD). Slope = 2078.28491; Intercept = 268.05856; r = 0.9993. Curve is weighted (1/x).

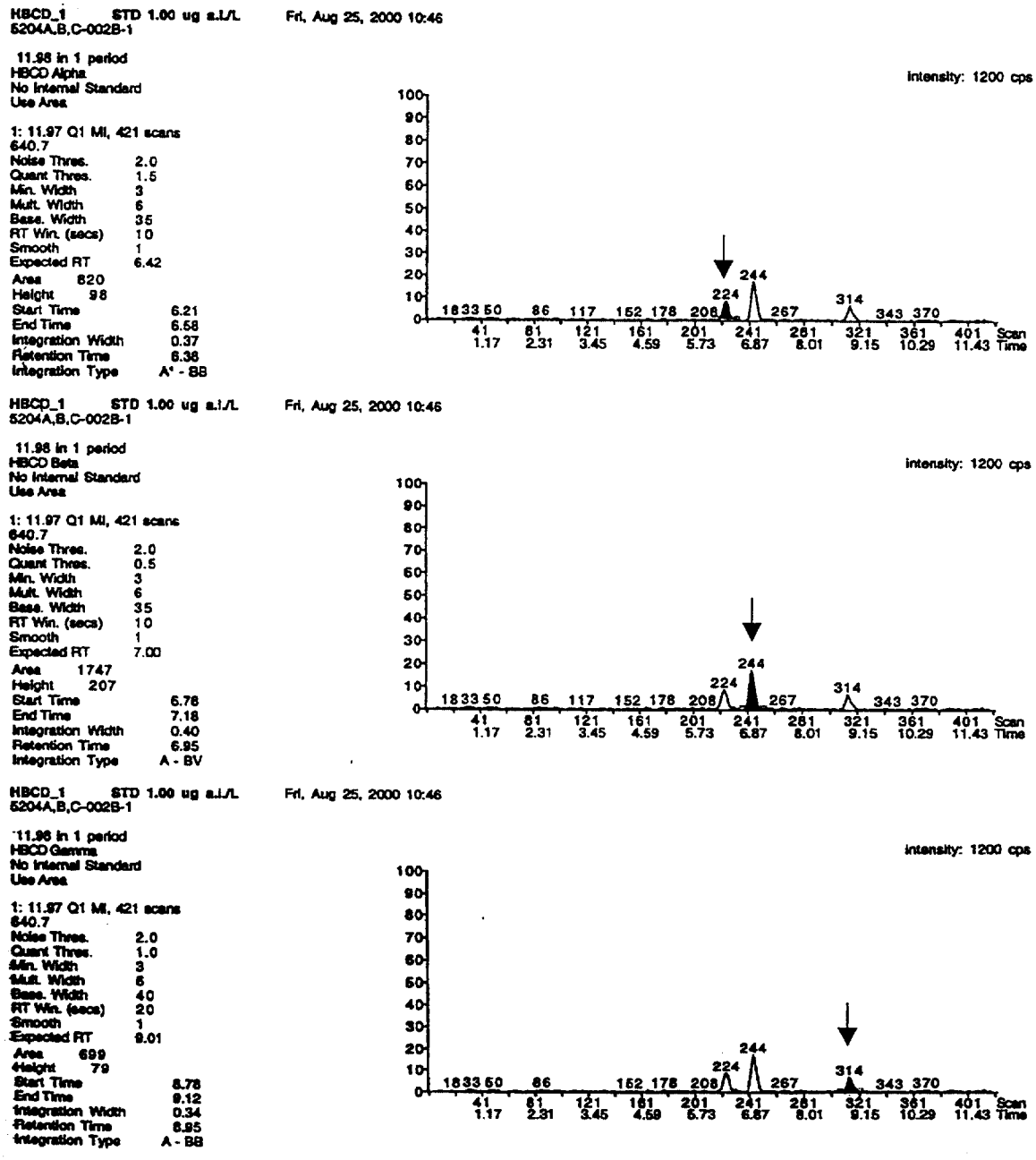


Figure 5. A representative ion chromatogram of a low-level combined alpha, beta, gamma HBCD diastereomer standard (1.00 µg a.i./L).

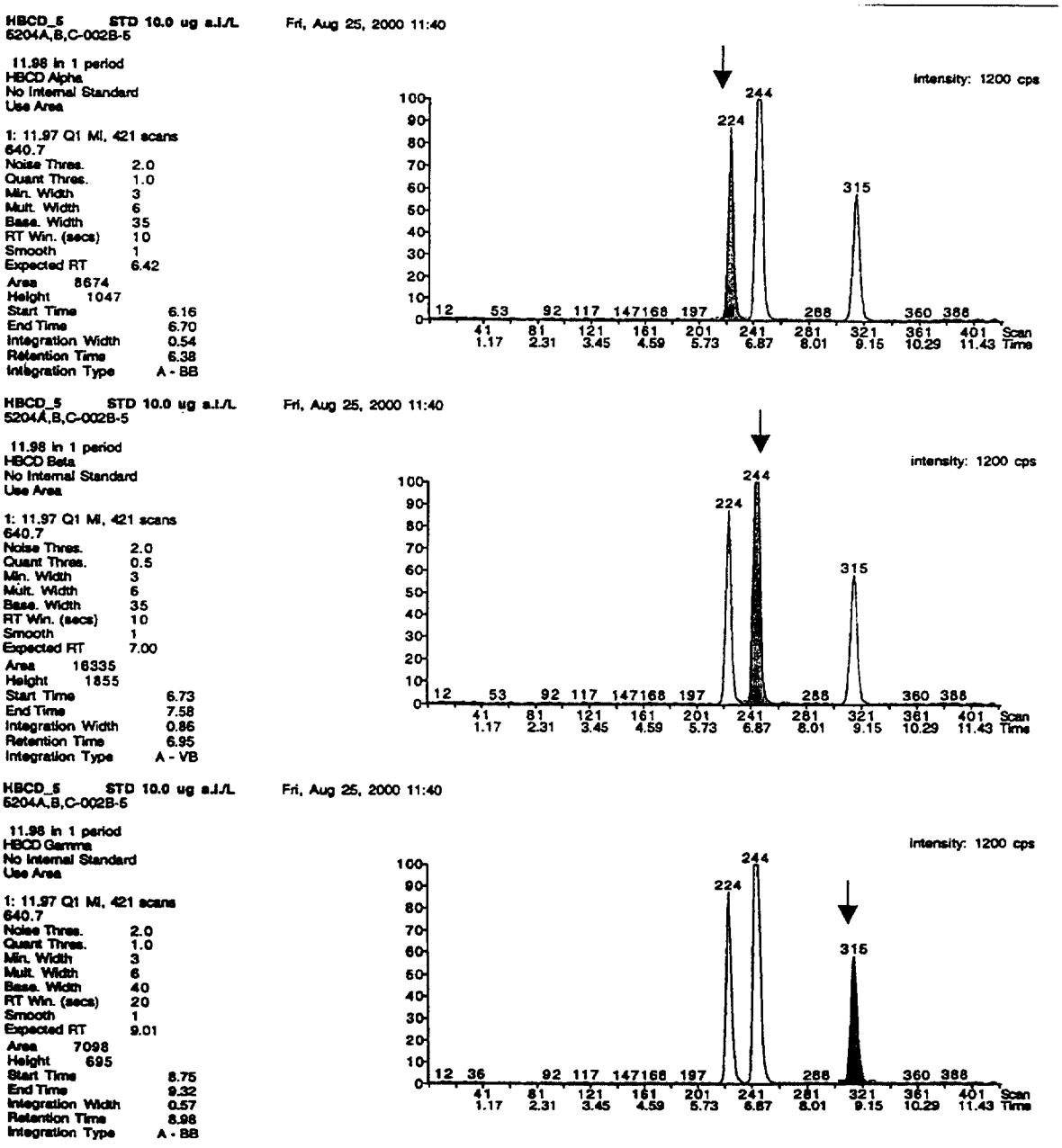


Figure 6. A representative ion chromatogram of a high-level combined alpha, beta, gamma HBCD diastereomer standard (10.0 µg a.i./L).

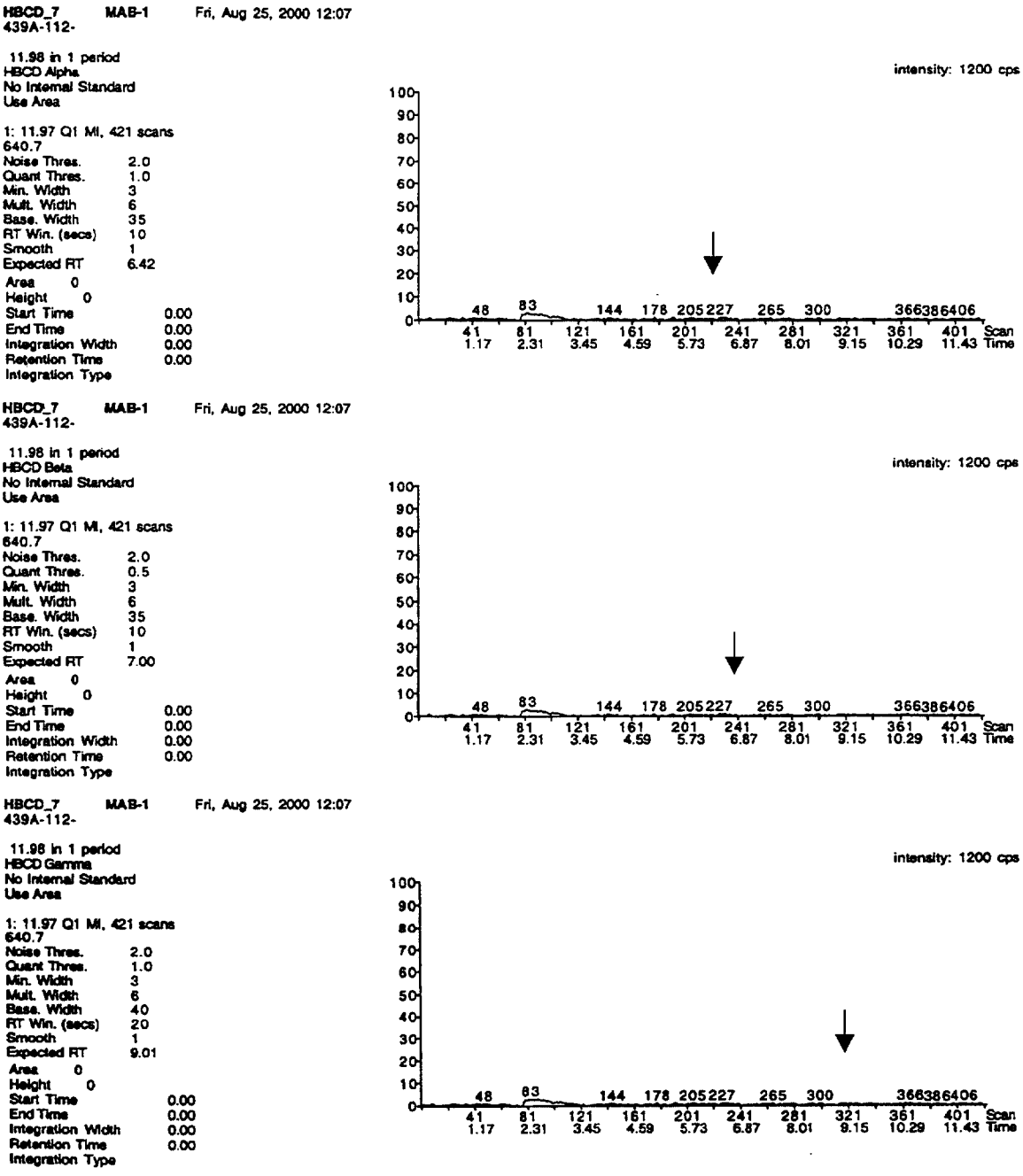


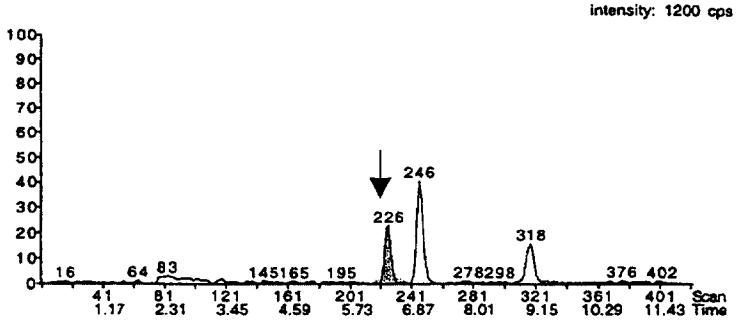
Figure 7. A representative ion chromatogram of a freshwater matrix blank sample (439A-112-MAB-1). The arrows indicate the retention times of alpha, beta, gamma HBCD diasteremers.

HBCD_8 MAS-1 Fri, Aug 25, 2000 12:20
439A-112-

11.98 in 1 period
HBCD Alpha
No Internal Standard
Use Area

1: 11.97 Q1 MI, 421 scans
640.7

Noise Thres.	2.0
Quant Thres.	1.0
Min. Width	3
Mult. Width	6
Base. Width	35
RT Win. (secs)	10
Smooth	1
Expected RT	6.42
Area	2212
Height	273
Start Time	6.27
End Time	6.58
Integration Width	0.31
Retention Time	6.44
Integration Type	A - BB

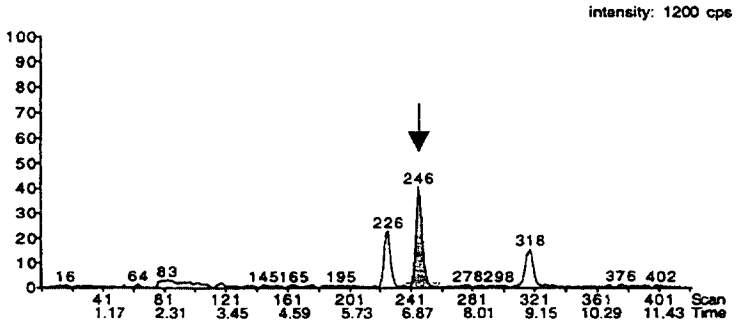


HBCD_8 MAS-1 Fri, Aug 25, 2000 12:20
439A-112-

11.98 in 1 period
HBCD Beta
No Internal Standard
Use Area

1: 11.97 Q1 MI, 421 scans
640.7

Noise Thres.	2.0
Quant Thres.	0.5
Min. Width	3
Mult. Width	6
Base. Width	35
RT Win. (secs)	10
Smooth	1
Expected RT	7.00
Area	4036
Height	486
Start Time	6.84
End Time	7.30
Integration Width	0.46
Retention Time	7.01
Integration Type	A - BB



HBCD_8 MAS-1 Fri, Aug 25, 2000 12:20
439A-112-

11.98 in 1 period
HBCD Gamma
No Internal Standard
Use Area

1: 11.97 Q1 MI, 421 scans
640.7

Noise Thres.	2.0
Quant Thres.	2.0
Min. Width	3
Mult. Width	6
Base. Width	40
RT Win. (secs)	20
Smooth	1
Expected RT	9.01
Area	1803
Height	180
Start Time	8.84
End Time	9.23
Integration Width	0.40
Retention Time	9.06
Integration Type	A* - BB

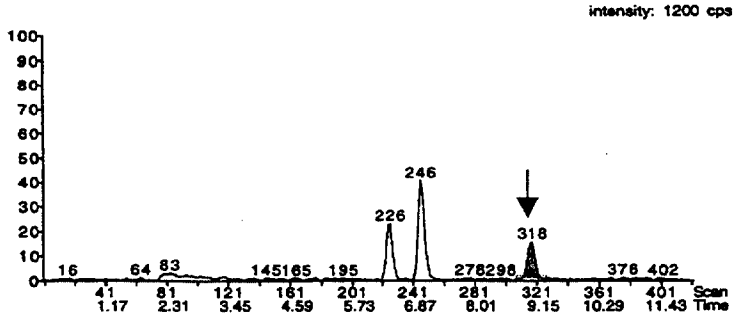


Figure 8. A representative ion chromatogram of a freshwater matrix fortification sample (439A-112-MAS-1, 0.100 µg alpha, beta, gamma HBCD diastereomer/L nominal concentration). The arrows indicate the retention times of alpha, beta, gamma HBCD diasteremers.

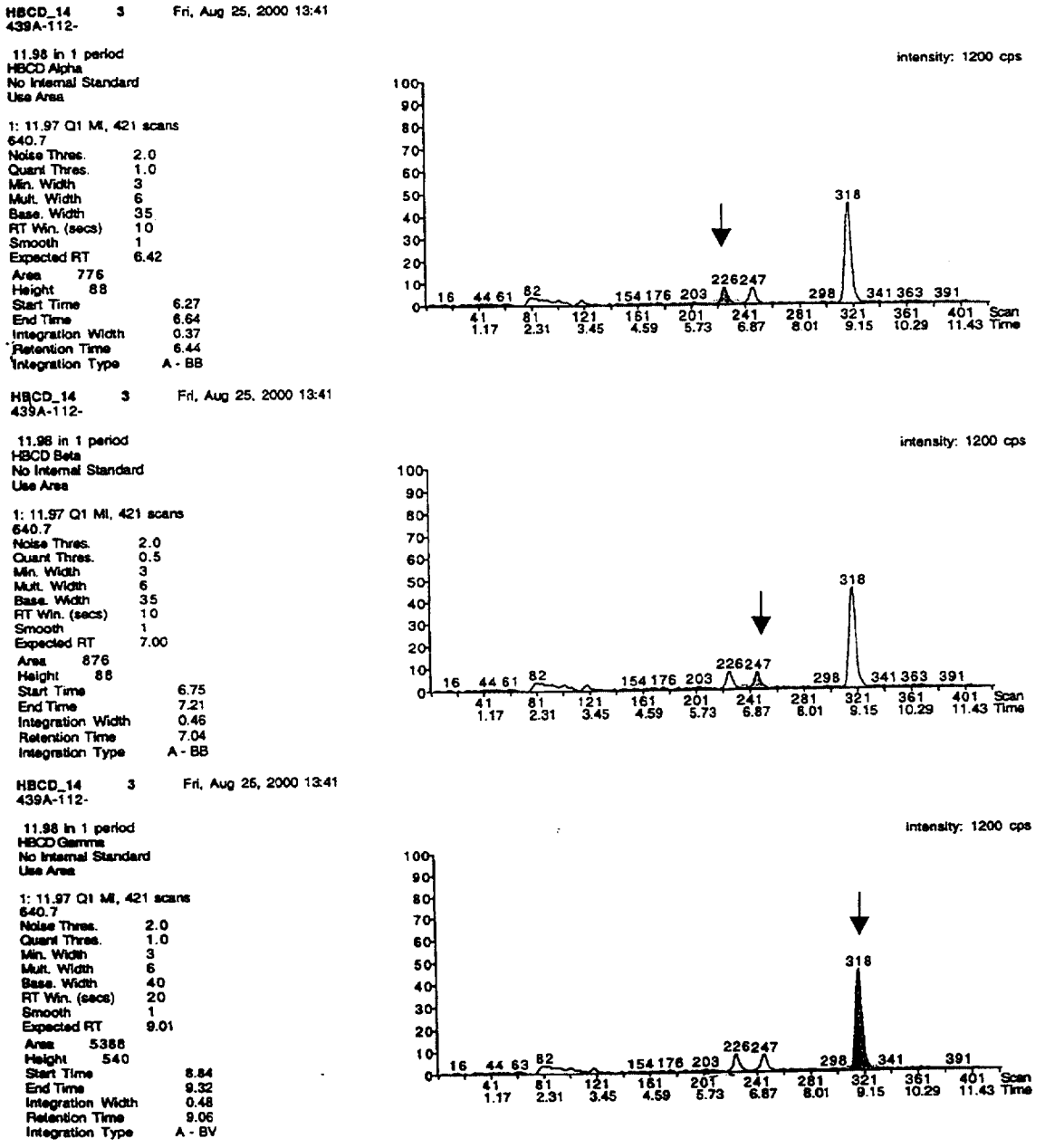


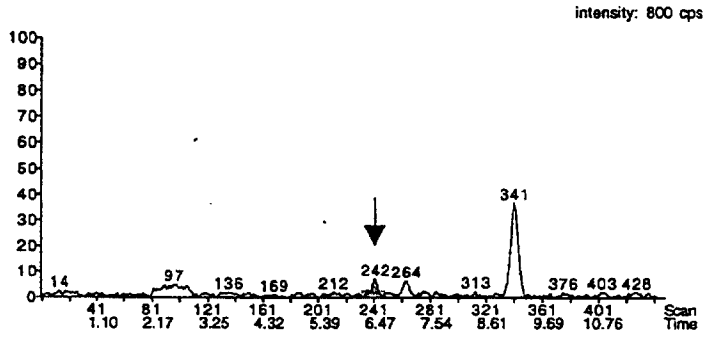
Figure 9. A representative ion chromatogram of a freshwater study sample at test initiation on Day 0 (439A-112-3; 0.43 ug HBCD test substance/L treatment level). The arrows indicate the retention times of alpha, beta, gamma HBCD diasteremers.

HBCD_14 94 Tue, Nov 21, 2000 14:20
439A-112-

11.98 in 1 period
HBCD Alpha
No Internal Standard
Use Area

1: 11.97 Q1 MI, 447 scans
640.7

Noise Thres.	2.0
Quant Thres.	1.5
Min. Width	3
Mult. Width	6
Base. Width	30
RT Win. (secs)	10
Smooth	1
Expected RT	6.57
Area	382
Height	48
Start Time	6.31
End Time	6.57
Integration Width	0.27
Retention Time	6.49
Integration Type	A* - BB

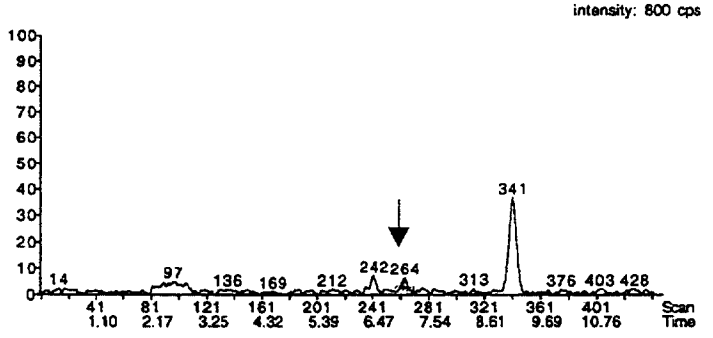


HBCD_14 94 Tue, Nov 21, 2000 14:20
439A-112-

11.98 in 1 period
HBCD Beta
No Internal Standard
Use Area

1: 11.97 Q1 MI, 447 scans
640.7

Noise Thres.	2.0
Quant Thres.	2.0
Min. Width	3
Mult. Width	6
Base. Width	30
RT Win. (secs)	10
Smooth	1
Expected RT	7.08
Area	259
Height	39
Start Time	6.98
End Time	7.16
Integration Width	0.19
Retention Time	7.08
Integration Type	A - BB



HBCD_14 94 Tue, Nov 21, 2000 14:20
439A-112-

11.98 in 1 period
HBCD Gamma
No Internal Standard
Use Area

1: 11.97 Q1 MI, 447 scans
640.7

Noise Thres.	2.0
Quant Thres.	1.0
Min. Width	3
Mult. Width	6
Base. Width	35
RT Win. (secs)	20
Smooth	1
Expected RT	9.31
Area	2961
Height	293
Start Time	8.86
End Time	9.31
Integration Width	0.46
Retention Time	9.15
Integration Type	A - BB

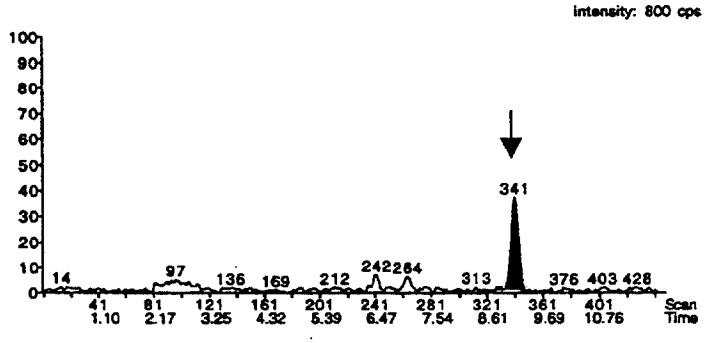


Figure 10. A representative ion chromatogram of a freshwater study sample at test termination on Day 88 (439A-112-94; 0.43 ug HBCD test substance/L treatment level). The arrows indicate the retention times of alpha, beta, gamma HBCD diasteremers.

Appendix 6

Fish Total Length (mm) at Day 29 Post-Hatch

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Fish Number															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
Negative Control	A	32	30	31	31	29	30	33	31	29	30	31	30	30	32	32	30.7
	B	34	35	30	30	30	32	30	30	34	32	29	31	30	31	31	31.3
	C	32	29	30	29	30	32	31	32	31	29	30	30	30	32	31	30.5
	D	30	31	31	31	32	29	29	33	30	31	31	29	30	31	29	30.5
Solvent Control	A	32	32	31	33	32	30	30	33	31	29	32	34	30	33	33	31.7
	B	34	34	31	31	31	32	32	30	29	29	32	30	30	30	31	31.1
	C	32	33	30	29	29	32	28	31	31	30	30	27	27	30	31	30.0
	D	29	29	31	29	33	33	30	31	31	32	33	31	30	27	32	30.7
0.25	A	32	32	26	31	27	31	32	30	30	31	29	29	31	30	29	30.0
	B	32	32	29	31	30	33	30	32	29	26	28	31	29	28	29	29.9
	C	30	33	32	31	30	30	30	31	28	31	30	31	31	30	33	30.7
	D	29	32	30	31	30	31	30	30	31	29	31	31	28	25	31	29.9
0.47	A	32	29	30	30	31	26	30	30	31	31	31	30	29	30	31	30.1
	B	32	31	31	31	30	32	31	31	28	32	31	28	31	29	30	30.5
	C	27	30	31	26	30	30	30	29	28	31	30	30	29	30	32	29.5
	D	29	31	31	29	31	32	31	31	28	31	29	31	32	30	30	30.4
0.83	A	31	31	30	30	29	32	31	30	27	30	28	29	34	31	30	30.2
	B	30	31	32	30	30	34	31	32	33	32	33	29	30	28	33	31.2
	C	35	33	30	30	33	32	31	30	30	31	26	31	29	28	32	30.7
	D	34	29	30	30	29	30	30	29	32	29	30	29	33	31	28	30.2
1.8	A	33	33	30	30	30	31	31	28	32	30	31	30	31	30	31	30.7
	B	31	28	30	26	30	30	31	32	30	30	29	29	30	27	30	29.5
	C	31	32	32	33	32	32	33	32	31	31	30	31	33	33	29	31.7
	D	32	31	31	30	30	32	32	31	30	29	32	31	31	32	33	31.1
3.7	A	31	31	31	32	29	33	31	28	31	29	31	32	31	33	35	31.2
	B	34	33	30	33	30	30	30	32	34	32	29	32	31	31	32	31.5
	C	31	34	30	35	31	32	32	31	31	29	31	28	32	30	31	31.2
	D	31	30	31	32	26	33	28	30	28	30	30	34	32	30	32	30.5

Appendix 7

Fish Total Length (mm) at Day 61 Post-Hatch

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Fish Number															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
Negative Control	A	49	52	49	50	48	46	51	50	51	48	52	51	51	53	54	50.3
	B	51	51	50	54	49	50	50	47	52	49	51	49	50	49	--	50.1
	C	49	48	48	49	53	49	51	48	52	51	53	48	51	52	48	50.0
	D	50	51	55	50	48	48	49	51	51	54	48	49	49	49	45	49.8
Solvent Control	A	47	50	48	48	48	52	48	51	52	47	51	52	51	49	47	49.4
	B	53	49	50	49	45	51	52	50	53	54	48	48	52	49	51	50.3
	C	53	53	52	49	44	53	48	50	51	50	51	50	51	55	--	50.7
	D	53	48	48	50	51	51	50	50	47	48	49	47	47	50	--	49.2
0.25	A	50	52	54	52	49	52	51	49	50	47	49	51	50	49	48	50.2
	B	49	44	51	47	53	51	48	46	49	49	52	49	48	52	47	49.0
	C	51	51	49	46	49	52	49	49	51	51	51	51	52	52	50	50.3
	D	48	49	51	49	48	54	51	47	48	49	52	44	49	47	49	49.0
0.47	A	48	51	50	48	48	51	49	51	50	50	49	53	48	--	--	49.7
	B	53	49	47	51	44	49	49	52	49	46	48	50	51	50	52	49.3
	C	51	50	51	48	49	51	50	48	48	47	49	49	49	51	53	49.6
	D	48	51	50	50	49	51	47	53	51	53	45	48	47	50	--	49.5
0.83	A	51	50	50	51	49	50	46	50	54	50	45	52	46	51	--	49.6
	B	50	49	50	48	42	50	48	51	52	48	53	50	52	50	49	49.5
	C	51	49	53	47	52	48	51	51	47	49	51	50	49	45	53	49.7
	D	48	50	49	51	53	49	49	47	49	50	46	50	53	50	48	49.5
1.8	A	53	48	50	48	48	47	47	50	45	51	52	49	51	51	50	49.3
	B	47	48	48	50	47	48	48	51	47	51	51	48	47	47	45	48.2
	C	49	51	49	52	49	51	48	49	48	48	50	51	50	52	52	49.9
	D	50	47	48	49	50	51	51	48	49	53	50	49	52	46	49	49.5
3.7	A	48	49	46	46	45	49	46	49	48	51	50	54	53	48	49	48.7
	B	48	48	51	50	53	52	47	52	48	53	50	50	50	52	49	50.2
	C	53	52	49	50	50	51	49	49	48	49	48	45	48	52	48	49.4
	D	53	50	53	48	51	50	47	48	49	50	47	51	49	51	51	49.9

-- = No measurement made due to mortality.

Appendix 8

Fish Wet Weight (g) at Day 61 Post-Hatch

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Fish Number															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
Negative Control	A	1.0415	1.2566	1.0505	1.0516	0.9457	0.7102	1.1875	0.7499	1.0671	0.9341	1.2728	1.0682	1.2454	1.3926	1.3323	1.0871
	B	1.2403	1.2490	1.1344	1.3624	1.0638	1.0643	1.1363	1.0171	1.2385	0.7366	1.0867	1.0195	1.1841	0.9879	--	1.1086
	C	1.0167	0.9844	0.9938	1.1045	1.2538	0.9737	1.1136	0.9862	1.3173	1.1563	1.3530	1.1484	1.1559	1.2081	0.9275	1.1129
	D	1.0773	1.2094	1.3711	1.0101	0.9547	1.0941	1.0679	1.2132	1.2672	1.3201	0.9407	1.0147	1.0376	1.0998	0.6905	1.0912
Solvent Control	A	1.0469	1.2294	1.0506	1.2797	0.9467	1.2979	1.0314	1.2386	1.2993	0.8279	1.1521	1.3356	1.1965	0.9857	0.9346	1.1235
	B	1.2637	1.0956	1.1179	1.0432	1.0006	1.1825	1.2666	1.0476	0.2735	1.4082	1.0034	1.0027	1.2714	0.9506	1.1620	1.1393
	C	1.3970	1.2974	1.3673	1.0444	0.8057	1.2529	1.0108	1.0606	1.1853	0.9597	1.2583	1.1617	1.1442	1.3630	--	1.1649
	D	1.4119	0.9748	0.9439	1.2070	1.3648	1.2138	1.1812	1.2347	0.9407	0.9250	1.0044	0.9795	0.8557	1.2213	--	1.1042
0.25	A	1.2177	1.3410	1.3757	1.2325	1.0976	1.2878	1.3842	1.1445	1.1820	1.0049	1.1396	1.2219	1.0482	0.9759	0.9813	1.1757
	B	0.9963	0.6941	1.2535	0.8797	1.3056	1.2552	1.1103	0.9724	1.1264	0.9422	1.3375	0.9486	1.0687	1.2292	0.9067	1.0684
	C	1.1564	1.1358	1.0627	0.8540	1.2097	1.2989	1.0095	0.9602	1.1183	1.2271	1.0244	1.2691	1.3879	1.2446	1.0407	1.1333
	D	1.0211	1.1759	1.1987	1.0412	1.0329	1.5873	1.1094	1.0822	1.0114	1.1397	1.2923	0.7348	1.0308	0.8675	0.9193	1.0830
0.47	A	1.1189	1.2162	1.2115	1.0080	1.0307	1.1659	1.1173	1.1492	1.1247	1.1050	1.0118	1.3078	1.0052	--	--	1.1209
	B	1.2647	1.0071	1.0645	1.3787	0.7352	1.0700	1.0843	1.2124	0.8702	0.9499	0.9230	1.1388	1.1874	1.2454	1.2376	1.0913
	C	1.1930	1.4010	1.3151	1.0638	1.0809	1.2254	1.1261	0.9543	1.0602	1.1076	1.1224	1.0939	1.1183	1.2747	1.3566	1.1662
	D	1.1639	1.2931	1.1128	1.0817	1.1155	1.1679	0.9378	1.3792	1.2301	1.3331	0.7279	1.0219	0.7569	1.1367	--	1.1042
0.83	A	1.2795	1.1503	1.1301	1.2067	1.0803	1.1480	0.8635	1.2294	1.3907	1.1657	0.8739	1.2617	0.8221	1.2240	--	1.1304
	B	1.1394	1.0979	1.1432	1.0833	0.6514	1.1096	1.0451	1.0993	1.2109	0.8854	1.2418	1.0794	1.0146	1.1227	0.9880	1.0608
	C	1.2358	1.0936	1.1968	0.9152	1.2389	0.9216	1.2916	1.1026	0.8520	1.0449	1.2062	1.0835	0.9970	0.8298	1.3126	1.0881
	D	0.8880	1.1117	0.9950	1.2953	1.1601	1.0305	1.0113	0.8430	1.0423	0.9665	0.7959	1.0339	1.3498	1.0520	0.9706	1.0364
1.8	A	1.3293	0.9896	1.0055	1.0162	1.0358	1.0190	0.9616	1.0899	0.9413	1.1575	1.1073	1.0281	1.1902	1.2370	0.9913	1.0733
	B	0.9491	1.0682	0.9120	1.0709	1.0106	0.9851	1.0179	1.1189	0.9550	1.1362	1.1043	0.8273	0.9677	0.8638	0.8920	0.9919
	C	1.0119	1.2010	1.0085	1.1869	1.0015	1.2661	1.0760	1.1581	0.9261	1.0501	1.0571	1.2040	1.0860	1.1089	1.1489	1.0994
	D	1.2042	0.9308	1.1840	1.1707	1.2071	1.2878	0.9507	1.0639	1.0566	1.3761	1.2213	1.0348	1.1782	0.8366	1.1967	1.1266
3.7	A	1.0422	1.0266	0.8027	1.0366	0.8471	1.0242	0.8768	1.0041	1.1254	1.1006	1.1317	1.4456	1.2103	1.0211	1.1575	1.0568
	B	1.0031	0.9959	1.2968	1.0704	1.2697	1.1524	0.9737	1.2839	1.0357	1.3720	1.1363	1.1655	1.0371	1.2176	1.0344	1.1363
	C	1.2960	1.2053	1.2480	1.2020	1.1486	1.0857	0.9551	1.0711	0.9238	1.0817	0.9935	0.9097	1.0314	1.1826	1.1206	1.0970
	D	1.3991	1.1584	1.1867	0.9598	1.1844	1.1367	1.0248	1.0360	1.0884	1.1072	0.6526	1.2186	1.0093	1.0818	1.1700	1.0943

-- = No measurement made due to mortality.

Appendix 9

Fish Dry Weight (g) at Day 61 Post-Hatch

Sponsor: American Chemistry Council's Brominated Flame Retardant Industry Panes
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Mean Measured Concentration (µg HBCD/L)	Replicate	Fish Number															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
Negative Control	A	0.2221	0.2684	0.2242	0.2272	0.1973	0.1491	0.2605	0.1581	0.2316	0.2102	0.2792	0.2323	0.2792	0.3044	0.2900	0.2350
	B	0.2694	0.2679	0.2400	0.3032	0.2252	0.2307	0.2515	0.2129	0.2750	0.1587	0.2365	0.2150	0.2665	0.2144	--	0.2405
	C	0.2180	0.2148	0.2153	0.2270	0.2699	0.2098	0.2447	0.2163	0.2858	0.2541	0.2953	0.2517	0.2286	0.2588	0.2000	0.2393
	D	0.2318	0.2658	0.3002	0.2160	0.2087	0.2310	0.2344	0.2755	0.2826	0.2935	0.2032	0.2144	0.2251	0.2349	0.1430	0.2373
Solvent Control	A	0.2233	0.2650	0.2310	0.2816	0.2048	0.2759	0.2167	0.2627	0.2851	0.1635	0.2559	0.2985	0.2628	0.2010	0.1931	0.2414
	B	0.2717	0.2361	0.2489	0.2287	0.2023	0.2545	0.2807	0.2281	0.2827	0.3129	0.2093	0.2185	0.2791	0.2050	0.2594	0.2479
	C	0.3024	0.2695	0.2849	0.2171	0.1513	0.2630	0.2173	0.2170	0.2637	0.2072	0.2770	0.2462	0.2386	0.2922	--	0.2462
	D	0.3023	0.1993	0.1876	0.2601	0.2865	0.2588	0.2470	0.2639	0.1985	0.1879	0.2170	0.2055	0.1791	0.2553	--	0.2321
0.25	A	0.2641	0.2884	0.2928	0.2590	0.2351	0.2800	0.3038	0.2460	0.2594	0.2137	0.2458	0.2696	0.2188	0.2123	0.2072	0.2531
	B	0.2130	0.1473	0.2721	0.1822	0.2777	0.2691	0.2361	0.2107	0.2389	0.2008	0.2926	0.2029	0.2321	0.2743	0.1891	0.2293
	C	0.2499	0.2419	0.2230	0.1838	0.2649	0.2806	0.2107	0.2135	0.2420	0.2705	0.2239	0.2812	0.3073	0.2585	0.2218	0.2449
	D	0.2244	0.2538	0.2573	0.2219	0.2256	0.3516	0.2468	0.2399	0.2192	0.2485	0.2914	0.1575	0.2328	0.1928	0.2050	0.2379
0.47	A	0.2413	0.2575	0.2577	0.2156	0.2206	0.2468	0.2415	0.2501	0.2392	0.2366	0.2109	0.2809	0.2109	--	--	0.2392
	B	0.2633	0.2151	0.2165	0.2997	0.1539	0.2271	0.2283	0.2645	0.1830	0.2027	0.1939	0.2407	0.2556	0.2764	0.2745	0.2330
	C	0.2490	0.3038	0.2854	0.2274	0.2354	0.2761	0.2364	0.1992	0.2319	0.2235	0.2458	0.2297	0.2437	0.2716	0.2913	0.2500
	D	0.2462	0.2717	0.2321	0.2246	0.2389	0.2435	0.1947	0.2822	0.2495	0.2765	0.1457	0.2179	0.1518	0.2367	--	0.2294
0.83	A	0.2653	0.2367	0.2329	0.2513	0.2189	0.2306	0.1805	0.2484	0.2952	0.2368	0.1805	0.2632	0.1668	0.2522	--	0.2328
	B	0.2472	0.2251	0.2467	0.2352	0.1234	0.2446	0.2223	0.2393	0.2639	0.1866	0.2681	0.2379	0.2226	0.2365	0.2204	0.2280
	C	0.2735	0.2322	0.2631	0.1971	0.2764	0.1878	0.2849	0.2306	0.1823	0.2261	0.2733	0.2370	0.2172	0.1759	0.2848	0.2361
	D	0.1768	0.2394	0.2085	0.2814	0.2536	0.2198	0.2093	0.1785	0.2228	0.2091	0.1685	0.2208	0.2970	0.2203	0.2089	0.2210
1.8	A	0.2881	0.2111	0.2111	0.2186	0.2280	0.2196	0.2042	0.3023	0.2031	0.2519	0.2373	0.2173	0.2624	0.2711	0.2105	0.2358
	B	0.1950	0.2241	0.1901	0.2193	0.2043	0.2087	0.2125	0.2357	0.1936	0.2357	0.2354	0.1684	0.2069	0.1796	0.1949	0.2069
	C	0.2108	0.2493	0.2142	0.2509	0.2119	0.2638	0.2298	0.2510	0.1933	0.2237	0.2264	0.2621	0.2361	0.2360	0.2445	0.2336
	D	0.2562	0.1907	0.2505	0.2421	0.2649	0.2702	0.2053	0.2302	0.2232	0.2977	0.2769	0.2263	0.2478	0.1747	0.2638	0.2414
3.7	A	0.2241	0.2199	0.1659	0.2249	0.1813	0.2255	0.1849	0.2207	0.2482	0.2294	0.2479	0.3272	0.2610	0.2208	0.2495	0.2287
	B	0.2071	0.2136	0.2830	0.2230	0.2714	0.2496	0.2088	0.2827	0.2202	0.2957	0.2430	0.2499	0.2204	0.2665	0.2260	0.2441
	C	0.2718	0.2569	0.2732	0.2589	0.2447	0.2303	0.2025	0.2319	0.1942	0.2323	0.2137	0.1926	0.2184	0.2565	0.2460	0.2349
	D	0.3055	0.2466	0.2516	0.2020	0.2499	0.2390	0.2237	0.2290	0.2419	0.2374	0.1335	0.2605	0.2135	0.2362	0.2458	0.2344

-- = No measurement made due to mortality.

Appendix 10

Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
2. Willard B. Nixon, Ph.D., Director, Analytical Chemistry
3. Cary A. Sutherland, Laboratory Supervisor
4. Kurt R. Drottar, Senior Biologist
5. Jon A. MacGregor, Scientist
6. Timothy L. Ross, Biologist

POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439E-104

AUTHORS:
Edward C. Schaefer
R. Scott Flaggs

STUDY INITIATION DATE: January 20, 2000

STUDY COMPLETION DATE: July 25, 2001

Submitted to:

Chemical Manufacturers Association's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

TITLE: Potential for Biotransformation of Radiolabelled Decabromodiphenyl Oxide (DBDPO) in Anaerobic Sediment

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439E-104

STUDY COMPLETION: July 25, 2001

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau), with the following exceptions:

Characterization of the test and reference substances was not conducted in compliance with Good Laboratory Practice Standards.

The stability of the test and reference substances under the conditions of storage at the test site was not conducted in compliance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

Edward C. Schaefer, B.S.
Manager, Biodegradation

July 25, 2001
DATE

SPONSOR/SUBMITTER:

Wendy Sherman

July 27, 2001
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Test Substance Preparation	March 9, 2000	March 10, 2000	July 7, 2000
Dosing	March 10, 2000	March 13, 2000	March 13, 2000
Preliminary Analytical Data Check	June 4 – 7, 2001	June 7, 2001	June 15, 2001
Analytical Draft Report	June 18, 2001	June 18, 2001	June 28, 2001
Biological Data and Draft Report	June 15, 18-22, 25-27, 2001	June 27, 2001	July 25, 2001
Final Report	July 25, 2001	July 25, 2001	July 25, 2001

Kimberly A. Hoxter

Kimberly A. Hoxter
Quality Assurance Representative

7-25-01
DATE

REPORT APPROVAL

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

TITLE: Potential for Biotransformation of Radiolabelled Decabromodiphenyl Oxide (DBDPO) in Anaerobic Sediment

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439E-104

STUDY DIRECTOR:

Edward C. Schaefer, B.S. — DATE: July 25, 2001
Manager, Biodegradation

MANAGEMENT:

Willard B. Nixon, Ph.D. — DATE: 7/25/01
Director, Analytical Chemistry

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APPENDICES

STUDY INFORMATION

Study Initiation Date:	January 20, 2000
Experimental Start Date (OECD):	March 06, 2000
Experimental Start Date (EPA):	March 10, 2000
Experimental Termination Date:	May 04, 2001
Study Completion Date:	July 25, 2001

Study Director:	Edward C. Schaefer
Sponsor:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Sponsor Representative:	Wendy Sherman
Study Personnel:	Edward C. Schaefer, B.S., Manager, Biodegradation Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants Willard B. Nixon, Ph.D., Director, Analytical Services Timothy Z. Kendall, M.S., Supervisor, Analytical Chemistry R. Scott Flaggs, B.S., Biologist Abul Siddiqui, B.A., Scientist Ken Chafey, B.S., Chemist Wendy Jenkins, B.S., Chemist Sheri Trumbull, Technologist

POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

EXECUTIVE SUMMARY

Wildlife International, Ltd. conducted an anaerobic mineralization test to determine the rate and extent of biotransformation and mineralization of commercial product and ^{14}C-labelled decabromodiphenyl oxide, nonvolatile test materials, under anaerobic conditions in a flooded sediment over a 32 week period. The freshwater sediment treatments employed in the mineralization test system consisted of a reference dosed with unlabelled and ^{14}C-labelled d-glucose and two DBDPO treatment groups dosed at nominal concentrations of 5 and 500 mg/kg DBDPO that were used to monitor the production of carbon dioxide ($^{14}CO_2$) and methane ($^{14}CH_4$). Two additional treatment groups were also prepared at 5 and 500 mg/kg. The additional treatment groups were not part of the mineralization (gas collection) system, but were utilized to monitor potential degradation of DBDPO using quantitative analytical methods.

Freshwater sediment samples and accompanying surface water were collected and stored at room temperature in an anaerobic chamber. Twenty test vessels were prepared in the anaerobic chamber one day prior to appropriate amounts of test or reference substance being introduced to their respective test chamber. A resazurin solution prepared using the decanted surface water was added to each vessel after dosing procedures were completed.

The eight test chambers apportioned to the mineralization test system were incubated in a water bath at room temperature (21 to 25°C) throughout the 224 day test period and the production of $^{14}CO_2$ and $^{14}CH_4$ was monitored over time and assayed for radioactivity by liquid scintillation counting (LSC).

Ten gram portions of the day-0 and week-32 dried sediments were extracted. The concentrations of DBDPO in the samples were determined using reversed phase high performance liquid chromatography (HPLC) with UV detection at 220 nm. The extracts were also profiled using a flow-through radioactivity detector (IN/US β-RAM Model 2B).

An average of 95% of the total activity added as radiolabelled glucose was recovered from the sediment in the reference test chambers. Of the recovered activity, 85% was recovered as $^{14}CO_2$ and $^{14}CH_4$ from the mineralization of the radiolabelled glucose and 10% was associated with the sediment. Mineralization of DBDPO was not observed. Less than 1% of the total activity added as decabromodi[U-^{14}C]phenyl ether was recovered as $^{14}CO_2$ and $^{14}CH_4$ indicating that no mineralization of the DBDPO had occurred.

Measured concentrations of DBDPO and radioactivity in the test sediments varied due to the composition of the individual sediment core. Sediments containing greater numbers of gravel/stones had proportionately less sediment and were a source of variability between replicates within and among the test sediments. Radiolabelled components eluting prior to ^{14}C-labelled DBDPO were detected in some of the week-32 5 mg/kg samples. Radiometric detection revealed 1 to 3 peaks in 9 of the 21 samples analyzed. HPLC analysis of a stock solution of the ^{14}C-labelled DBDPO test material also exhibited components eluting prior to the ^{14}C-deca congener using radiometric detection.

Concentrations of DBDPO in the test sediments at the start and conclusion of the study were evaluated using two approaches. In the first approach, seven replicate samples of each test sediment were analyzed by the HPLC-UV procedure. Average measured DBDPO concentrations in the 5 mg/kg sediments on day-0 and week-32 were 6.64 ± 0.70 mg/kg and 6.51 ± 2.15 mg/kg, respectively. Average measured DBDPO concentrations in the 500 mg/kg sediments on day-0 and week-32 were 543 ± 77 mg/kg and 612 ± 158 mg/kg, respectively. Statistical analysis of the data using ANOVA was carried out in order to assess whether the measured concentrations of DBDPO at the start and conclusion of the 32 week test period were significantly different. The F test concluded that the difference between the mean measurements on day-0 and week-32 were not statistically significant (5 mg/kg $P = 0.9525$; 500 mg/kg $P = 0.6555$). In the second approach, measured DBDPO concentrations were converted to a DBDPO mass based on the actual dry weight of the sediment and compared to the mass of DBDPO added at test initiation. For the 5 mg/kg sediments, the mean differences between the measured mass and the added mass in day-0 and week-32 samples were 0.123 and 0.127 mg, respectively. For the 500 mg/kg sediments, the mean differences between the measured mass and the added mass in day-0 and week-32 samples were 65.0 and 0.96 mg,

respectively. The difference between the measured mass and mass added was analyzed using a paired t-test. The differences between the DBDPO mass weighed into the test chamber on day-0 and the DBDPO mass calculated using the measured DBDPO concentration at week-32 were also found not to be statistically different (5 mg/kg P = 0.9672; 500 mg/kg P = 0.3764).

Based on the results of this study, DBDPO was neither biotransformed nor mineralized under anaerobic conditions in a flooded sediment over a 32 week period.

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for the Chemical Manufacturers Association's (currently American Chemistry Council) Brominated Flame Retardant Industry Panel at the Wildlife International, Ltd. facility in Easton, Maryland. The experimental phase of the test was conducted from 06 March 2000 to October 2000. Original raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 439E-104 in the archives located on the Wildlife International, Ltd. site.

OBJECTIVES

The objective of the study was to determine the rate and extent of biotransformation of a nonvolatile radiolabelled decabromodiphenyl oxide test material under anaerobic conditions in a flooded sediment. Anaerobic sediment was dosed with ^{14}C-labelled decabromodiphenyl oxide (DBDPO) and incubated under anaerobic conditions. Evolved $^{14}CO_2$ and $^{14}CH_4$ were trapped continuously using a trapping/combustion train and quantified by liquid scintillation counting (LSC). The total amount of radioactivity recovered as $^{14}CO_2$ and $^{14}CH_4$ were expressed as a percent of the amount of radioactivity dosed. Sediment was analyzed for the test material and screened to observe possible biotransformation products.

EXPERIMENTAL DESIGN

The test contained one reference and two treatment groups that were used to monitor the production of $^{14}CO_2$ and $^{14}CH_4$. The reference group contained two replicate test chambers and was dosed with a combination of unlabelled and ^{14}C-labelled glucose at a concentration of 5 mg/kg. The two treatment groups contained 3 replicate test chambers and were used to evaluate the biotransformation of the test substance at 5 and 500 mg/kg. The test chambers were incubated at ambient room temperature and the production of $^{14}CO_2$ and $^{14}CH_4$ was monitored over a period of 32 weeks. The headspace of the test chambers was continuously purged with nitrogen and then passed through two CO_2 traps. The effluent gas from the CO_2 traps was channeled through a quartz column packed with cupric oxide at 800°C in a tube furnace to combust methane to CO_2. The gas exiting the combustion column was passed through two additional CO_2 traps. CO_2 traps were periodically collected and analyzed for radioactivity by liquid scintillation counting (LSC). At the end of the 32-week test period, samples from each of the reference and treatment group test chambers

were analyzed for DBDPO and biotransformation products (if any). The results from the reference sediments were used to provide information about potential contaminants present in the sediment prior to the start of the test.

Six additional treatment chambers were prepared at both 5 and 500 mg/kg but were not attached to the headspace gas collection system. Samples from the additional test chambers were to be analyzed for DBDPO and metabolites (if any) only if significant degradation of DBDPO was observed at the end of the 32 -week test period. No statistically significant degradation was observed.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Potential for Biotransformation of Radiolabelled Decabromodiphenyl Oxide (DBDPO) in Anaerobic Sediment" (Wildlife International, Ltd. Protocol Number 439/111099/MAS/SUB439) (Appendix 5). Test methods were based on the procedures described by Nuck and Federle (1).

Reference and Test Substances

Following is a description of the nonlabeled reference substance used in this study.

Name:	Dextrose, Anhydrous
Wildlife International, Ltd. ID Number:	5194
CAS Number:	50-99-7
Manufacturer:	EM Science
Lot Number:	128547-M031100
Physical Description:	White, granular powder
Purity:	100%
Storage Conditions:	Ambient

Following is a description of the labeled reference substance used in this study.

Name:	D-glucose-UL-^{14}C
Wildlife International, Ltd. ID Number:	5189

CAS Number:	50-99-7
Manufacturer:	Sigma chemical
Lot Number:	109 H 9400
Physical Description:	Aqueous solution
Radiochemical Purity:	99.3%
Radiochemical Concentration:	0.095 mCi/mL
Specific Activity:	7.3 mCi/mmol
Storage Conditions:	Frozen

The nonlabelled test substance consisted of a composite of decabromodiphenyl oxide samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International, Ltd. Identification Number
Great Lakes Chemical Corporation	5480DH24A	October 26, 1995	3460
Albemarle Corporation	4449-1N	December 20, 1995	3518
Bromine Compounds Ltd.	950289	January 30, 1996	3547

The composite test substance was assigned Wildlife International, Ltd. identification number 3578 and was stored under ambient conditions. Subsamples of the composite test substance were shipped to Albemarle Corporation for characterization and homogeneity analyses. The analyses were performed on March 13, 1996. Results of the analyses indicated the composite test substance was homogeneous and contained the following components:

Octabromodiphenyl oxide	0.04%
Nonabromodiphenyl oxide	2.5%
Decabromodiphenyl oxide	97.4%

The radiolabelled form of the test material was received from Nycomed Amersham on December 14, 1999, and was assigned Wildlife International, Ltd. identification number 5160. The test substance was identified on the label as decabromodi[U-^{14}C]phenyl ether; CAS No. 1163-19-5.

A specific activity of 19 mCi/mmol, a molecular weight of 959.8, and a radiochemical purity of 96.8% were reported by the manufacturer. The test substance was stored under frozen conditions.

Reference Substance Preparation and Administration

A primary stock solution of the nonlabelled form of d-glucose was prepared on March 07, 2000 at a nominal concentration of 1 g/L in NANO®pure water.

The radiolabelled/nonlabelled reference stock solution of d-glucose was prepared by combining 9.8 mL of the nonlabelled primary stock solution, 105 µL of the radiolabelled form of the reference material (d-glucose-UL-^{14}C) and 100 µL of NANO®pure water. The activity of the combined reference stock as measured by LSC was 0.94 µCi/mL (94% of nominal activity). The total concentration of d-glucose in the radiolabelled/nonlabelled reference stock solution was calculated to be 1.0 mg/mL. This solution was stored under refrigeration.

On March 10, 2000 a sufficient amount of the radiolabelled/nonlabelled reference stock solution was added to the sediment reference vessels to achieve a nominal concentration of 5 mg/kg. The reference stock was administered by volumetric addition. The total amount of radioactivity added to each reference vessel ranged from 2.24 to 2.39 µCi.

Test Substance Preparation and Administration

On March 09, 2000 the radiolabelled test substance decabromodi[U-^{14}C]phenyl ether was quantitatively transferred into an appropriate container using 10.0 mL of tetrahydrofuran (THF) and vortexed well to ensure homogeneity. The activity of the primary stock as measured by LSC was 4.6 µCi/mL (92% of the nominal activity level). Based on the measured and specific activities, the concentration of test material in the primary stock was determined to be 0.23 mg/mL.

The radiolabelled stock solution of the test material was administered by volumetric addition to dried sediment. The dried sediment containing the labelled test substance was allowed to stand overnight before being added to the test chambers to facilitate the dissipation of the THF solvent. The total amount of radioactivity added to each test vessel was 1.84 µCi. To assess the effects of the

solvent on the test system, an equivalent volume of THF was administered to dried sediment and handled in an identical manner before being added to each reference chamber.

The nonlabelled form of the test substance (DBDPO) was administered to each test chamber by direct weight addition. Sufficient radiolabelled and nonlabelled forms of the test substance were added to 9 test vessels to achieve a nominal concentration of 5 mg/kg in each of these test chambers. The labeled/nonlabelled test substances were administered to an additional 9 test vessels to achieve a nominal test concentration of 500 mg/kg.

Test Inoculum

Sediment and accompanying surface water were collected from the Schuykill River, Valley Forge, Pennsylvania on March 06, 2000. Upon collection, the redox potential of the sediment was measured and determined to be –284 mV. Prior to use, the surface water was decanted from above the sediment and placed in a separate container. The surface water and sediment were characterized by Agvise Laboratories, Inc. (Northwood, North Dakota). The sediment characterization included pH, % organic matter (Walkley-Black), cation exchange capacity (Ca, Mg, Na, K & H), disturbed bulk density, % sand-silt-clay, USDA textural class, and water holding capacity (1/3 bar). The surface water characterization included pH, nitrate-nitrogen, sulfate-sulfur, and total phosphorus. A copy of the characterization reports is included in Appendix 4. The collected sediment cores were stored at room temperature in an anaerobic chamber for 4 days. The average percent moisture of the freshwater sediment was 26.0%. The decanted surface water was stored under refrigeration during this time. A 0.2 mg resazurin/L solution was prepared using the surface water.

Mineralization Test Apparatus and Conditions

An illustration of the mineralization apparatus that included flow controllers for nitrogen (N_2) and oxygen (O_2), incubation vessels, water bath, check valves, carbon dioxide (CO_2) traps, tube furnace, combustion tubes, and trapping train is presented in Figure 1. The headspace gases within each of the test chambers attached to the mineralization test system were continuously purged with a flow of nitrogen (approximately 5 mL/min.) and passed through a gas collection system consisting of two sets of CO_2 traps and a combustion apparatus. The displaced gases were initially passed through one empty bottle followed by two bottles each containing 100 mL of 1.5N KOH (CO_2 trapping

solution) followed by another empty bottle. The gas was combined with a flow of oxygen (approximately 2 mL/min) and channeled through a quartz column that was packed with cupric oxide and maintained at approximately 800°C in a tube furnace to combust methane to CO_2. The gas exiting the combustion column was passed through an empty bottle followed by two additional CO_2 traps. The test chambers were incubated in a water bath at room temperature. Water temperatures were measured each working day and ranged from 21 to 25°C.

Preparation of the Test Chambers

All test vessels were graduated 500 mL glass media bottles and were identified by project number, test substance ID, test concentration, and vessel number. The test chambers were transferred to an anaerobic chamber. Sufficient sediment to reach the 300 mL graduation was added to each chamber. Each sediment was added to the appropriate test chamber in a manner consistent with maintaining the integrity of the sediment column structure (i.e. bottom of column on bottom of vessel, top of column on top). The numbers of bacteria are typically highest in surface sediments and decrease rapidly within sediments at greater depths (2). The test chambers were capped then removed from the anaerobic chamber and weighed. All test chambers were returned to the anaerobic chamber then uncapped and allowed to equilibrate overnight. After the equilibration period, the appropriate amounts of test or reference substance were added to their respective test chamber. Each sediment system was mixed using a wooden applicator so that the test and reference substances were distributed throughout the top 1 inch of sediment. The lower part of the wooden applicator was broken off and left in the test chamber. Approximately 10 mL of the resazurin/surface water solution was added to each chamber. The chambers apportioned to the mineralization test apparatus (duplicate reference vessels, triplicate treatment vessels at 5 mg/kg, and triplicate treatment vessels at 500 mg/kg) were then sealed and transferred out of the anaerobic chamber and connected to the gas collection system. Two of the additional treatment vessels dosed at 5 mg/kg and two dosed at 500 mg/kg were each acidified with 10 mL of concentrated H_2SO_4, sealed, transferred out of the anaerobic chamber and stored under refrigeration. The additional test chambers (4 treatment chambers dosed at 5 mg/kg and 4 treatment chambers dosed at 500 mg/kg) that were not connected to the gas collection system or acidified were stoppered with a gas trap and incubated at approximately 22 °C within the anaerobic chamber.

Sample Collection and Analysis

The 1st CO_2 trap of each set (before and after combustion apparatus) was removed once a week over the 32-week test period. Three replicate 1 mL aliquots of each trap were analyzed for radioactivity by LSC. The 2nd trap in each set was moved to the 1st position and a new trap was placed in the 2nd trap spot.

Two chambers each from the 5 and 500 mg/kg treatments that were prepared and stoppered with a gas trap for incubation in the anaerobic chamber were acidified using 10 mL of concentrated sulfuric acid on Weeks 13 and 26. Acidified test chambers were stored under refrigeration until analysis (if any). Samples from the additional test chambers were to be analyzed for DBDPO and metabolites (if any) only if significant degradation of DBDPO was observed at the end of the 32-week test period.

Test Termination

On Day 224 of the test period, the contents of the test chambers attached to the mineralization apparatus were acidified by the addition of 10 mL of concentrated sulfuric acid to terminate biological activity. The chambers were purged for approximately 24 hours. After purging, pH measurements of the sediments were taken. Since the measured pH of each sediment was > 2.0, an additional 10 mL of concentrated sulfuric acid was added to the chambers. The chambers were purged for approximately 24 hours longer and additional pH measurements were taken. The measured pH of each chamber was < 2.0 and the remaining traps were sampled and analyzed by LSC.

The contents of the reference and treatment group test chambers attached to the mineralization apparatus were transferred to glass drying pans and air dried at room temperature. Individual dried sediments were transferred to mill jars and subsequently homogenized using a "roller type" jar mill. The contents of the mill jars were tumbled at the highest speed of the jar mill. Aliqouts of the dried sediments were analyzed for DBDPO and screened for metabolites (if any).

Analytical Method

All analytical glassware was pre-rinsed with tetrahydrofuran (THF). Recovery samples were prepared by directly fortifying 10 g aliquots of sediment with the appropriate DBDPO stock solution.

Unfortified sediment served as the matrix blanks. Ten grams of sediment sample (not corrected for dry weight) was combined with 100 mL of THF within an 8-oz., French square bottle. The bottle was capped, secured to a shaker table and the contents were mixed for approximately 15 minutes at a setting of 250 rpm. Following this period, the sample was centrifuged for approximately 5 minutes at 1500 rpm. The extract was gently poured through a pledget of glass wool contained in a glass funnel and the filtrate collected in a roundbottom flask ensuring that the solids remained in the French square bottle. The extraction was repeated with an additional 100 mL of THF; the sample was shaken, centrifuged and the extract combined in the roundbottom flask with the initial extract. The THF extract was rotary evaporated to approximately 2-3 mL which was quantitatively transferred to a 25-mL volumetric flask. The final volume of the extract was adjusted to 25 mL with THF. Each final extract was subsequently diluted (as appropriate) using 50% tetrahydrofuran: 50% water, (v:v), filtered through a 0.45 µm Acrodisc and transferred to an autosampler vial. Concentrations of DBDPO in the samples were determined using high performance liquid chromatography (HPLC) with UV and radiometric detection using a Hewlett-Packard Model 1090 High Performance Liquid Chromatograph (HPLC) equipped with either a Waters 486 variable wavelength detector, an HP 1100 variable wavelength detector or a Jasco 975 detector operated at 220 nm and an INUS β-RAM radiometric detector. Chromatographic separations were effected using a Zorbax phenyl analytical column (250 mm x 4.6 mm, 5 µm particle size). The instrument parameters are summarized in Table 1 and a method flow chart is provided in Figure 2.

Calibration Curve and Limit of Quantitation

External calibration standards of DBDPO were prepared in 50% THF : 50% water and ranged in concentration from 0.0500 to 0.500 mg/L. Standards were analyzed prior and subsequent to the samples and at a minimum after every fifth sample. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. A representative calibration curve is presented in Figure 3. The concentration of DBDPO in the samples was determined by substituting the peak area responses into the linear regression equation. Representative chromatograms of low and high calibration standards are shown in Figures 4 and 5, respectively.

The method limit of quantitation for the analysis was arbitrarily defined as 1.25 µg/g as calculated from the product of the low standard (0.0500 mg/L) and the dilution factor of the matrix blank (25.0).

Matrix Blank

In addition to the samples, a matrix blank was analyzed with each sample set to determine the presence or absence of chromatographic interferences. No interferences were observed at or above the LOQ (1.25 µg/g); see Appendix 2. A representative chromatogram of a sediment matrix blank is presented in Figure 6.

Fortification Samples

Two quality control samples were fortified and concurrently processed with each sample set as specified in the analytical method. The sediment samples were fortified to reflect nominal concentrations of either 5.00 or 500 µg/g. These samples yielded mean recoveries of 91.2 and 89.8%, respectively; see Appendix 2. A chromatogram of a quality control sediment fortification is presented in Figure 7.

Example Calculation

Sample number: 439E-104-9F

Nominal Concentration: 5.00 µg/g

Mass Extracted: 10.0 grams

Initial Final Volume: 25.0 mL

Secondary Dilution: 1:10

Dilution Factor: 25.0

Peak Area: 133.45070

Slope: 596.68

Intercept: 0.4290

$$\text{DBDPO } (\mu g/g) = \frac{(\text{Peak area} - (\text{Y-intercept})) \times \text{dilution factor}}{\text{Slope}}$$

$$\text{DBDPO } (\mu g/g) \text{ in sample} = \frac{(133.45070 - 0.4290) \times 25.0}{596.68} = 5.57\ \mu g/g$$

$$\text{Percent Recovery} = \frac{\text{DBDPO } (\mu g/g) \text{ in sample}}{\text{Nominal DBDPO concentration } (\mu g/g)}$$

$$\text{Percent Recovery} = \frac{5.57\ \mu g/g}{5.00\ \mu g/g} = 111$$

Mass Balance Determination

Replicate samples of the dried sediments were combusted using a Packard Model 307 Oxidizer. The samples from the oxidizer were then assayed using a Packard Model 2500 TR liquid scintillation counter to determine the amount of radioactivity associated with the dried sediments.

Calculations

The amount of $^{14}CO_2$ & $^{14}CH_4$ evolved was calculated using the following equations (A&B):

A) $$\frac{(CO_2\ dpm \times 100)}{initial\ dpms} = \%\ radioactivity\ recovered\ as\ CO_2$$

B) $$\frac{(CH_4\ dpm \times 100)}{initial\ dpms} = \%\ radioactivity\ recovered\ as\ CH_4$$

where:

Initial radioactivity = total dpms added to test chamber, and

CO_2 (or CH_4) dpms = mean of replicates of 1 mL trapping solution

Note: $^{14}CH_4$ was actually detected as $^{14}CO_2$ after combustion.

The radioactivity associated with the sediment was calculated using the following equation (C):

C) $$\frac{sediment\ dpms}{initial\ dpms} \times 100 = \%\ radioactivity\ remaining\ on\ sediment$$

where:

sediment dpms = mean of replicate dried sediment samples (dpms/g) x dried sediment wt (g).

A total mass balance will be calculated using the following equation:

Total Mass Balance = A + B + C

The measured DBDPO concentrations were converted to a DBDPO mass based on the actual dry weight of the sediment and the measured mass was compared to the mass of DBDPO added at test initiation.

Treatment of Results

The average measured DBDPO concentrations of the day-0 and week-32 test sediments were statistically analyzed. In addition, the differences between the DBDPO mass weighed into the test chambers on day-0 and the DBDPO mass calculated using the measured DBDPO concentration at week-32 were statistically analyzed.

RESULTS

Summaries of ^{14}C gas evolution based on the total activity administered at test initiation are presented in Appendix 1. The results of the mass balance determination based on the amount of radioactivity dosed are presented in Table 2. An average of 95% of the total activity added as radiolabelled glucose was recovered from the sediment in the reference test chambers. Of the recovered activity, 85% was recovered as $^{14}CO_2$ and ^{14}CH from the mineralization of the radiolabelled glucose and 10% was associated with the sediment. Mineralization of DBDPO was not observed. Less than 1% of the total activity added as decabromodi[U-^{14}C]phenyl ether was recovered as $^{14}CO_2$ and ^{14}CH indicating that no mineralization of the DBDPO had occurred. Averages of approximately 96% and 98% of total activity added were recovered from the 5 and 500 mg/kg day-0 sediments, respectively. The recoveries from the day-0 sediments were consistent with that of the radiolabelled glucose dosed reference sediments. Averages of approximately 131% and 123% of total activity added were recovered from the 5 and 500 mg/kg week-32 sediments, respectively.

Ten gram portions of the day-0 and week-32 dried sediments were extracted two times with tetrahydrofuran (THF). Final extracts were concentrated and subsequently diluted, as appropriate, using 50% tetrahydrofuran: 50% water, (v:v), filtered through a 0.45 µm Acrodisc and transferred to

an autosampler vial. The concentrations of DBDPO in the samples were determined using reversed phase high performance liquid chromatography (HPLC) with UV detection utilizing a system consisting of a Hewlett-Packard Model 1090 High Performance Liquid Chromatograph (HPLC) equipped with either a Waters 486 variable wavelength detector, an HP 1100 variable wavelength detector or a Jasco 975 detector operated at 220 nm. The extracts were also profiled using a flow-through radioactivity detector (IN/US β-RAM Model 2B). Chromatographic profiles of a ^{14}C-labelled DBDPO stock solution and the test sediment can be seen in Figures 9 and 10, respectively. Chromatographic separations were effected using a Zorbax phenyl analytical column (250 mm x 4.6 mm, 5 µm particle size). Residual activity associated with extracted solids was measured using a Packard Model 307 oxidizer to evaluate the efficiency of the extraction process.

Seven replicate samples of each test sediment were analyzed by the HPLC-UV procedure. A summary of the HPLC analysis results is presented in Table 3. Average measured DBDPO concentrations in the 5 mg/kg sediments on day-0 and week-32 were 6.64 ± 0.70 mg/kg and 6.51 ± 2.15 mg/kg, respectively. Average measured DBDPO concentrations in the 500 mg/kg sediments on day-0 and week-32 were 543 ± 77 mg/kg and 612 ± 158 mg/kg, respectively. A representative chromatogram of a test sample is shown in Figure 8. A statistical test (ANOVA) was carried out in order to assess whether the measured concentrations were significantly different (3). The differences between the days were analyzed using a nested ANOVA, with vessels nested within days. The denominator of the F test for effect of day was the ANOVA mean square for vessels within days. The F test concluded that the difference between the mean measured concentrations of DBDPO on day-0 and week-32 were not statistically significant.

Measured concentrations of DBDPO and radioactivity in the test sediments varied due to the composition of the individual sediment core. Sediments containing greater numbers of stones had proportionately less sediment and were a source of variability between replicates within and among the test sediments. Measured DBDPO concentrations were converted to a DBDPO mass based on the actual dry weight of the sediment and compared to the mass of DBDPO added at test initiation. The calculated mass of DBDPO in each test chamber is presented in Table 4. For the 5 mg/kg sediments, the mean differences between the measured mass and the added mass in day-0 and week-32 samples were 0.123 and 0.127 mg, respectively. For the 500 mg/kg sediments, the mean differences between

the measured mass and the added mass in day-0 and week-32 samples were 65.0 and 0.96 mg, respectively. The difference between the measured mass and mass added was analyzed using a paired t-test. The differences between the DBDPO mass weighed into the test chambers on day-0 and the DBDPO mass calculated using the measured DBDPO concentration at week-32 were also found not to be statistically different.

Chromatographic profiles of day-0 and week-32 test sediments are presented in Figures 10 and 11, respectively. Radiolabelled components eluting prior to ^{14}C-labelled DBDPO were detected in some of the week-32 5 mg/kg samples. Radiometric detection revealed 1 to 3 peaks in 9 of the 21 samples analyzed (Figure 12). HPLC analysis of a stock solution of the ^{14}C-labelled DBDPO test material also exhibited components eluting prior to the ^{14}C-deca congener using radiometric detection (Figure 9).

Based on the results of this study, DBDPO was neither biotransformed nor mineralized under anaerobic conditions in a flooded sediment over a 32 week period.

REFERENCES

1. **Nuck, B.A., Federle, T.W.** 1996. *A Batch Test for Assessing the Mineralization of ^{14}C-Radiolabeled Compounds under Realistic Anaerobic Conditions*. Environmental Science & Technology.

2. **Wetzel, R. G.** 1975. Limnology. P592-593. W.B. Saunders Company, Philadelphia, Pa.

3. **SAS Institute, Inc.** 1989. SAS/STAT User's Guide , Version 6, Fourth Edition, Volume 1, Cary, NC, SAS Institute, Inc.

Table 1

HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with either a Hewlett-Packard Model 1100 Variable Wavelength Detector, a Waters 486 variable wavelength detector or a Jasco 975 detector and a INUS β-RAM radiometric detector
ANALYTICAL COLUMN:	Zorbax phenyl (250 mm x 4.6 mm, 5 μm particle size)
STOP TIME:	20.0 minutes
FLOW RATE:	1.00 mL/minute
SCINTILLANT FLOW:	3.00 mL/minute
OVEN TEMPERATURE:	40°C
MOBILE PHASE A:	45% CH_3CN : 55% H_20 : 0.1% H_3PO_4
MOBILE PHASE B:	95% CH_3CN : 5% H_20 : 0.1% H_3PO_4
GRADIENT ELUTION PROFILE:	

Time (minutes)	% A	% B	Flow Rate (mL/min.)
0.01	50.0	50.0	1.00
2.00	50.0	50.0	1.00
10.0	0.0	100	1.00
16.0	0.0	100	1.00
16.1	50.0	50.0	1.00
20.0	50.0	50.0	1.00

INJECTION VOLUME:	150 μL
DBDPO PEAK RETENTION TIME:	15.0 minutes
PRIMARY ANALYTICAL WAVELENGTH:	220 nm

Table 2

Results of Mass Balance Determination (Based on Radioactivity Dosed at Test Initiation)[1]

Test/Reference Substance	Nominal Conc. (mg/kg)	% Recovered as $^{14}CO_2$	% Recovered as $^{14}CH_4$	Recovered % as ^{14}C-Gas	% ^{14}C Remaining with Solids	Total ^{14}C Recovery (%)
Glucose	5	67.2 ± 2.1	18.1 ± 1.1	85.4 ± 3.1	9.5 ± 4.9	94.9 ± 1.8
DBDPO	5	0.4 ± 0.04	0.4 ± 0.04	0.86 ± 0.06	129.9 ± 24.1	130.8 ± 24.1
DBDPO	500	0.4 ± 0.03	0.4 ± 0.06	0.80 ± 0.05	122.5 ± 7.9	123.3 ± 7.9

[1] Calculations were performed using Excel 2000. Small variances may exist for certain percentage values displayed in the table as a result of rounding of significant figures.

Table 3

Measured DBDPO Concentrations

Nominal Test Concentration (mg/kg)	Description	DBDPO Concentration (mg/kg dry weight)							Sample Mean	Sample Standard Deviation	Group Mean
		Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6	Rep. 7			
5.00	Day 0	6.17[1]	5.88	6.08	6.15	6.08	5.57	6.07	6.00	0.21	6.64
5.00	Day 0	7.53[1]	7.30	7.40	6.75	7.04	7.51	7.37	7.27	0.28	
500	Day 0	540[1]	382	538	502	537	499	397	485	68	543
500	Day 0	608[1]	599	616	588	567	602	625	601	19	
0.0 (Control)	Week 32	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA	< LOQ
0.0 (Control)	Week 32	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ		
5.00	Week 32	9.24[1]	9.26	9.17	9.24	9.15	9.04	9.28	9.20	0.08	6.51
5.00	Week 32	5.90[1]	6.68	6.25	6.10	5.89	5.92	6.67	6.20	0.35	
5.00	Week 32	4.15[1]	4.08	4.60	3.83	4.73	3.78	3.73	4.13	0.40	
500	Week 32	840[1]	809	673	720	725	761	776	758	57	612
500	Week 32	703[1]	693	569	724	637	639	694	666	54	
500	Week 32	444[1]	337	454	440	363	403	447	413	46	

[1] Average of duplicate analyses.

Table 4

Recovery of DBDPO and Total Radioactivity Based on Mass Conversion[1]

Nominal Test Concentration (mg/kg)	Description	Average Percent Recovered of Total Amount DBDPO Added[2]	Average Percent Recovered of Total Radioactivity Added[3]
5	Day 0	91.4	94.1
5	Day 0	101	98.9
500	Day 0	65.6	95.4
500	Day 0	84.5	101
5	Week 32	107	103
5	Week 32	103	146
5	Week 32	74.5	144
500	Week 32	105	124
500	Week 32	95.4	115
500	Week 32	70.7	131

[1]-Calculations performed using Excel 2000 in full precision mode.
[2]-[(measured DBDPO concentration x dry weight of sediment)/mass of DBDPO added]x100
[3]-[(dpm added-dpm evolved) x dry weight of sediment/activity added]x100.

Figure 1

Mineralization Apparatus

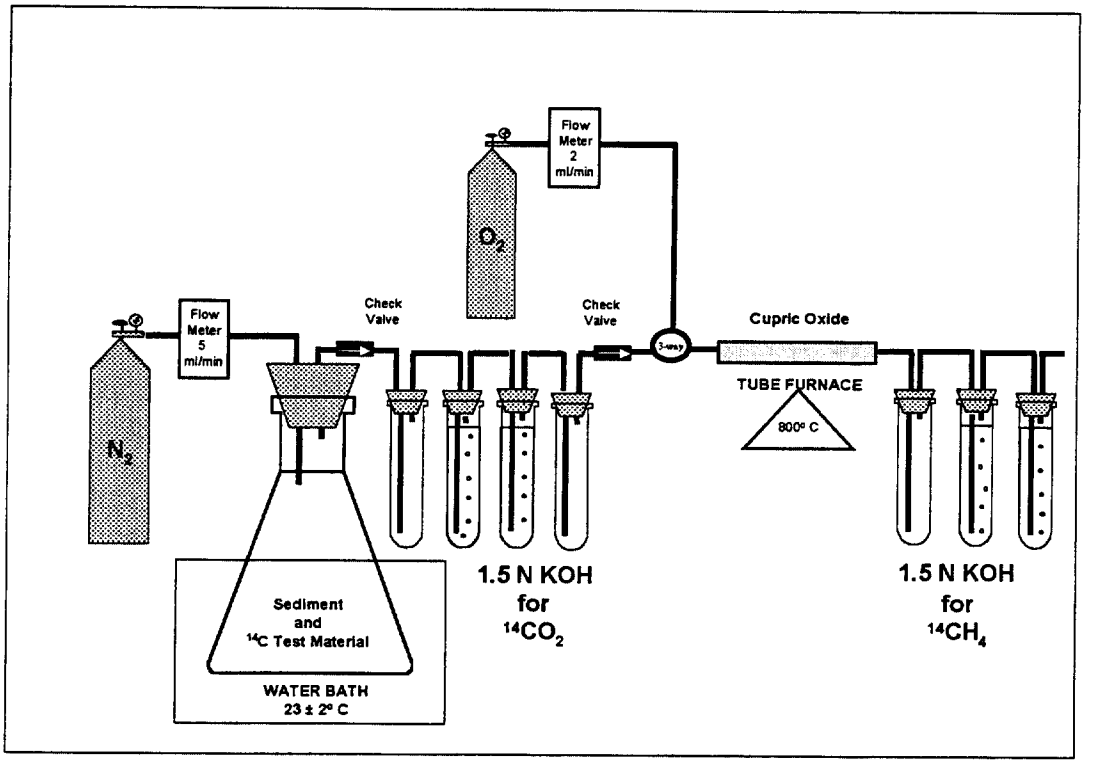


Figure 2

Analytical Method Flowchart for the Analysis of DBDPO in Sediment

METHOD OUTLINE FOR THE PROCESSING OF DBDPO IN SEDIMENT

Pre-rinse all glassware with tetrahydrofuran.

↓

Prepare recovery samples by directly fortifying 10.0 g of sediment (contained in 8-oz. French square bottles) with the appropriate DBDPO stock solution. Unfortified sediment will serve as the matrix blank. For test samples, weigh 10.0 g of each into 8-oz French square bottles.

↓

To each recovery and study sample, add 100 mL of tetrahydrofuran. Seal samples and place on a shaker table for ~15 minutes at a setting of 250 rpm.

↓

Centrifuge samples ~5 minutes at a setting of 1500 rpm.

↓

Pour the extracts through glass wool contained in glass funnels into roundbottom flasks.

↓

Repeat the extraction procedure using an additional 100-mL of tetrahydrofuran and combine the extracts in their respective roundbottom flasks.

↓

Rotary evaporate the samples to ~2-3 mL.

↓

Quantitatively transfer the concentrated extract using tetrahydrofuran to the appropriate size volumetric flask.

↓

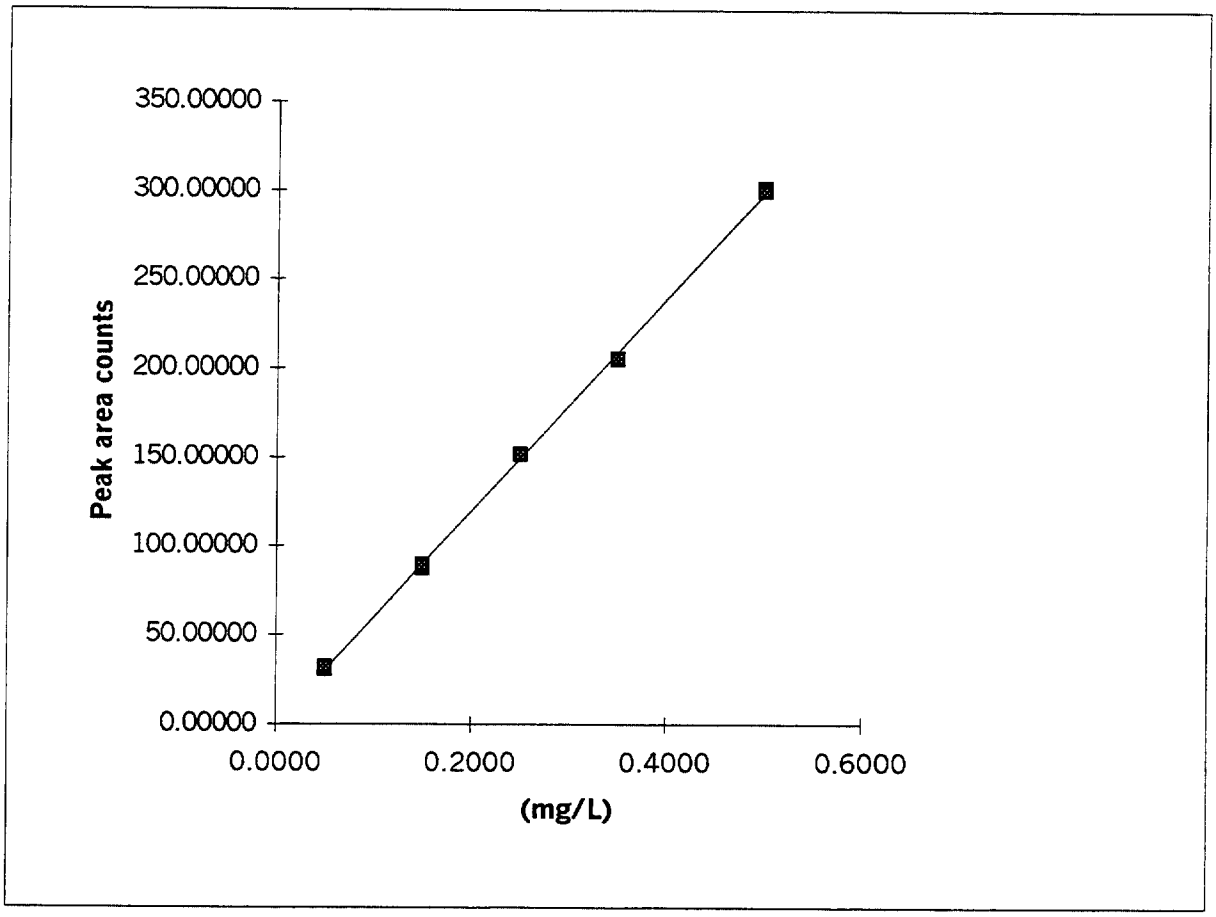
Perform secondary dilutions where appropriate using 50% tetrahydrofuran : 50% water.

↓

Filter aliquots from each extract through 0.45 acrodiscs directly into autosampler vials and submit for HPLC/UV analysis.

Figure 3

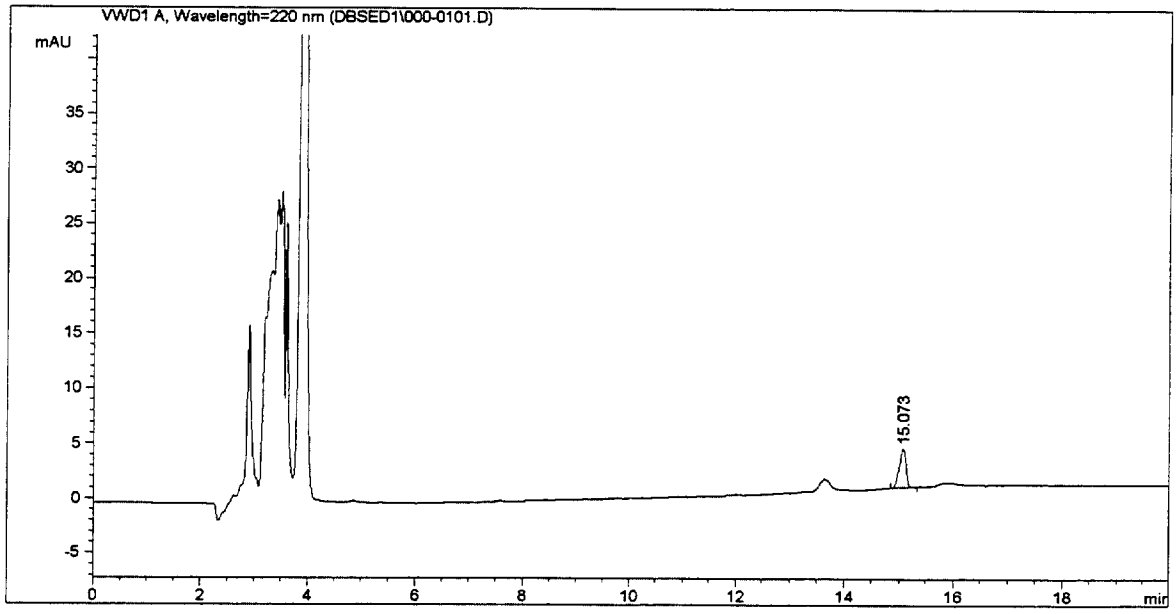
Representative Calibration Curve for DBDPO



Slope = 596.68; Y-Intercept = 0.4290; R^2 = 0.9995

Figure 4

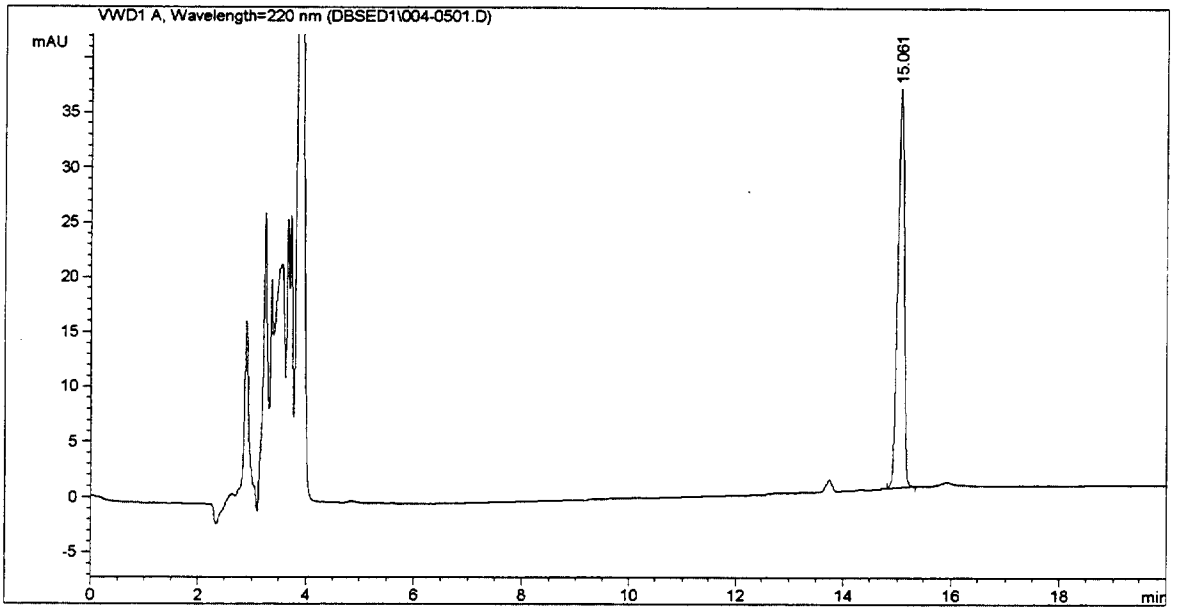
Representative Chromatogram of a Low-level DBDPO Calibration Standard



Nominal concentration: 50.0 µg/L

Figure 5

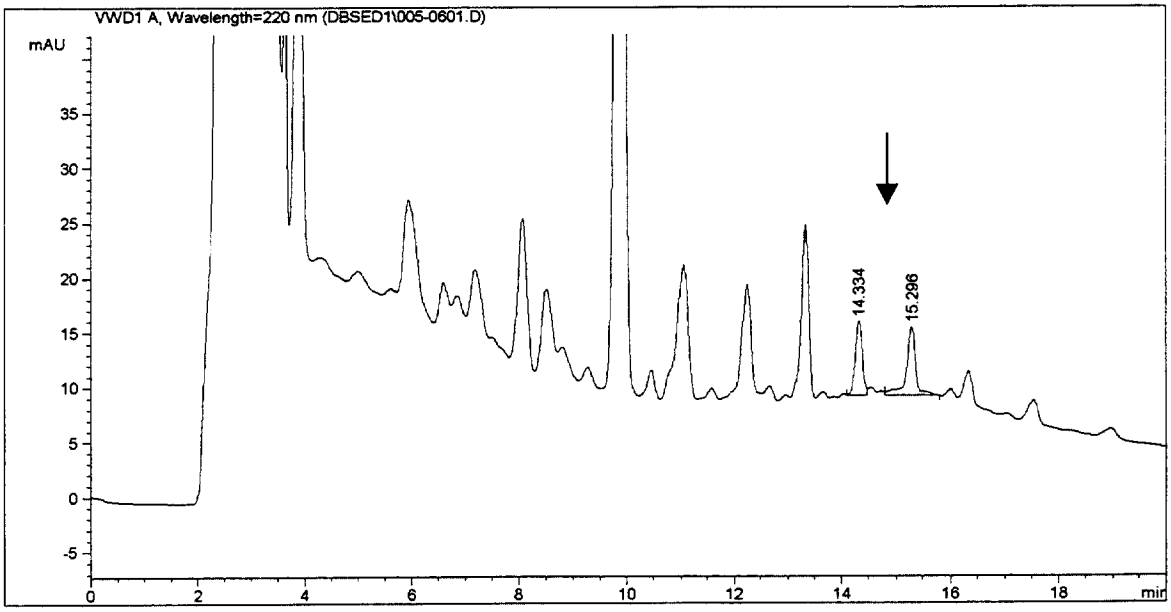
Representative Chromatogram of a High-level DBDPO Calibration Standard



Nominal concentration: 500 µg/L

Figure 6

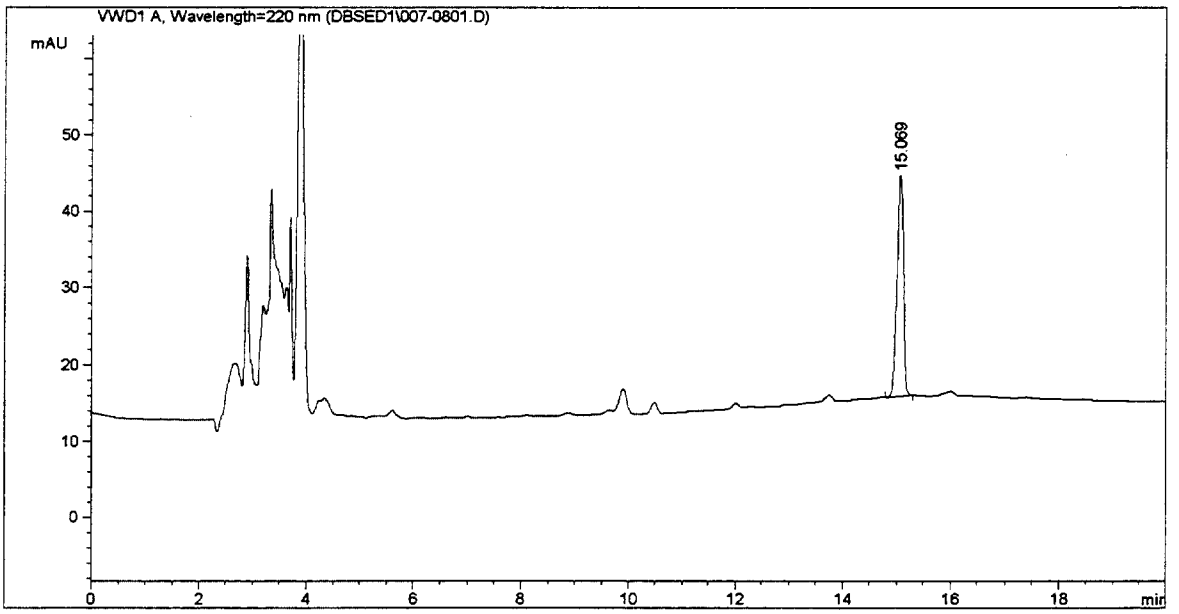
Representative Chromatogram of a Matrix Blank Sample



Sample number 439E-104-MAB-3. The arrow indicates the approximate retention time of DBDPO.

Figure 7

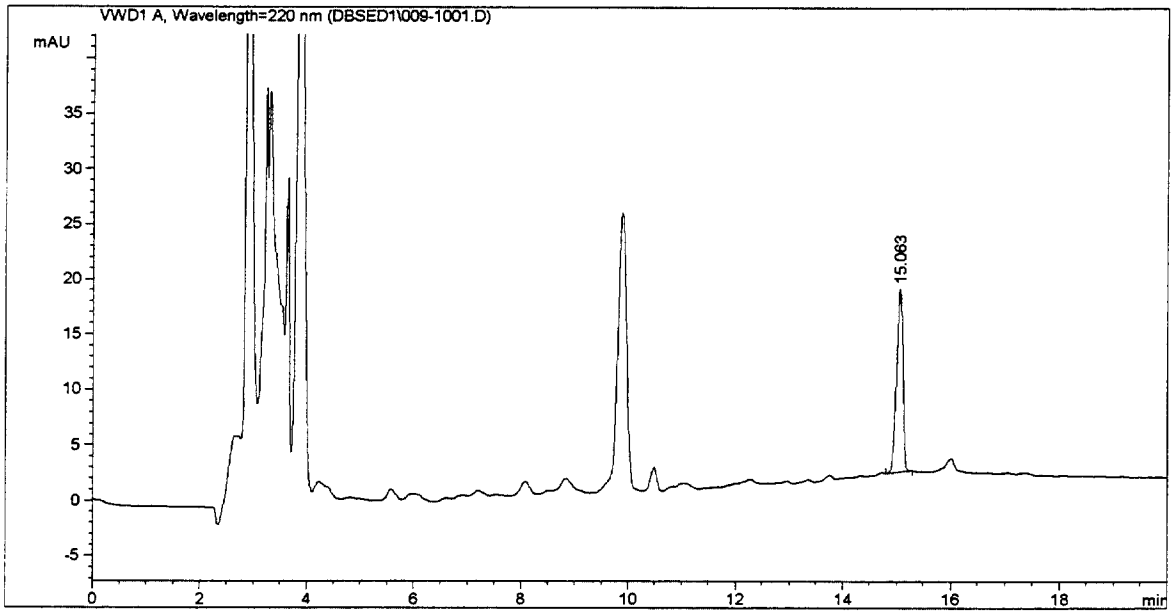
Representative Chromatogram of a Matrix Fortification Sample



Sample number 439E-104-MAS-6; 500 mg/kg nominal concentration

Figure 8

Representative Chromatogram of a Test Sample

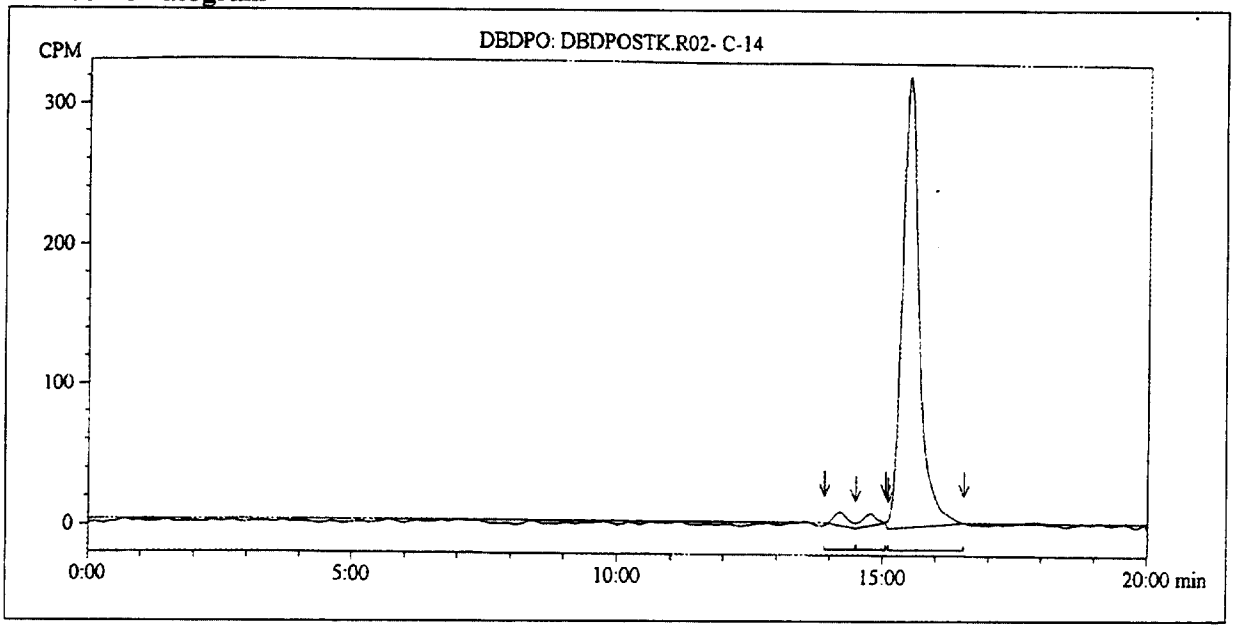


Sample number 439E-104-9B; 0 Hour; 5.00 mg/kg nominal concentration

Figure 9

Chromatographic Profile of ^{14}C-labelled DBDPO Stock Solution

Radiochromatogram



UV Chromatogram

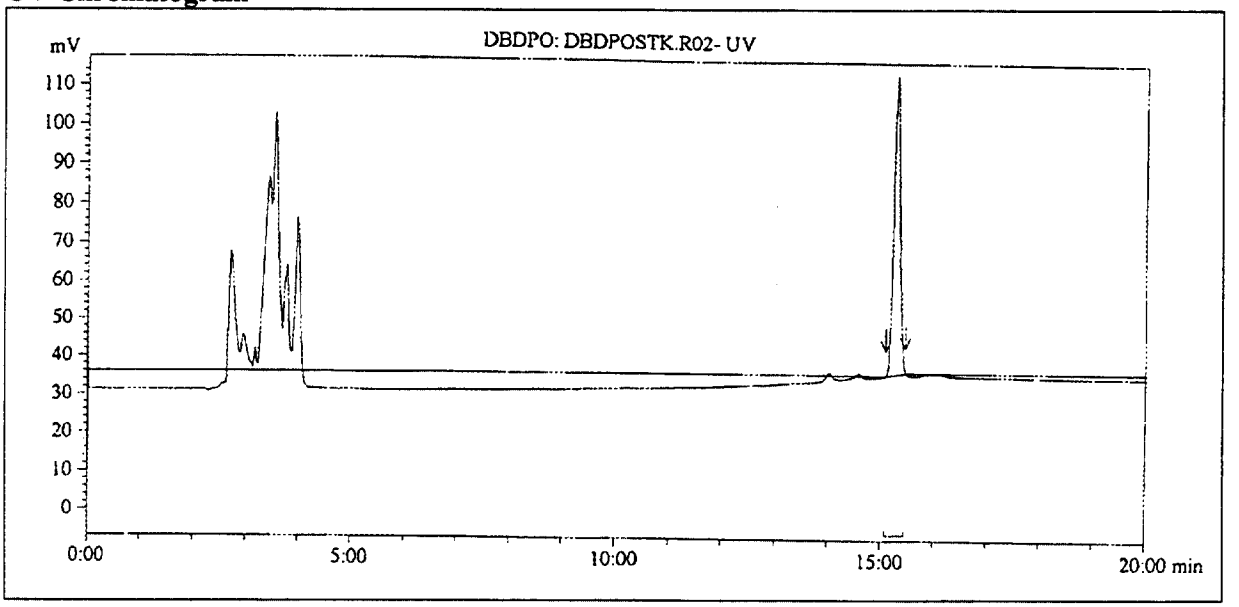
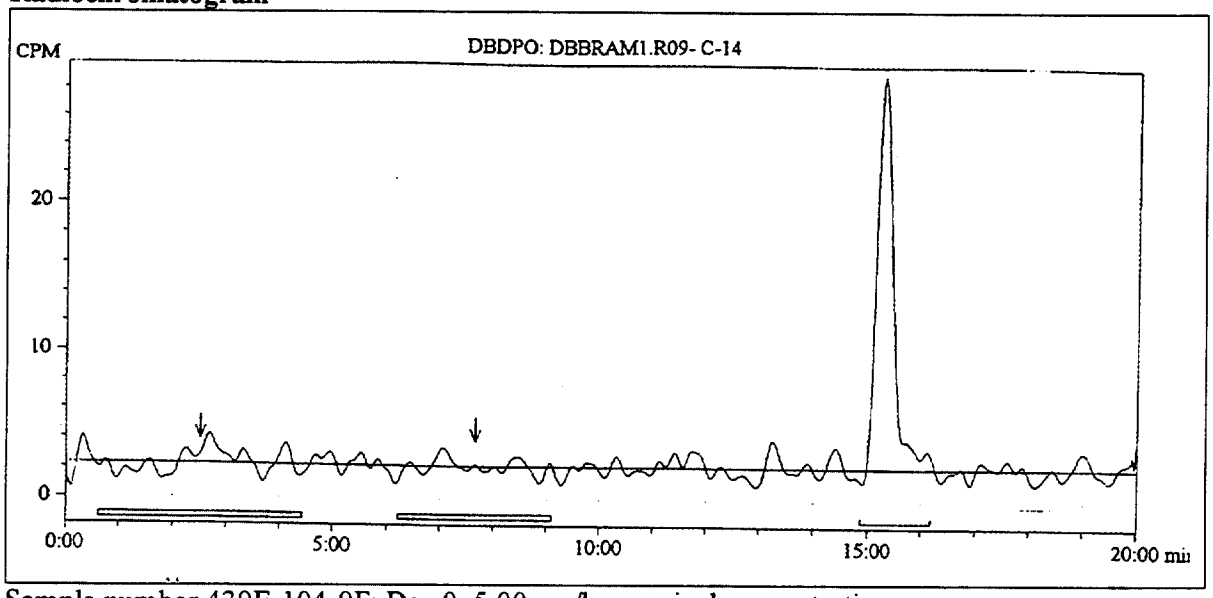


Figure 10

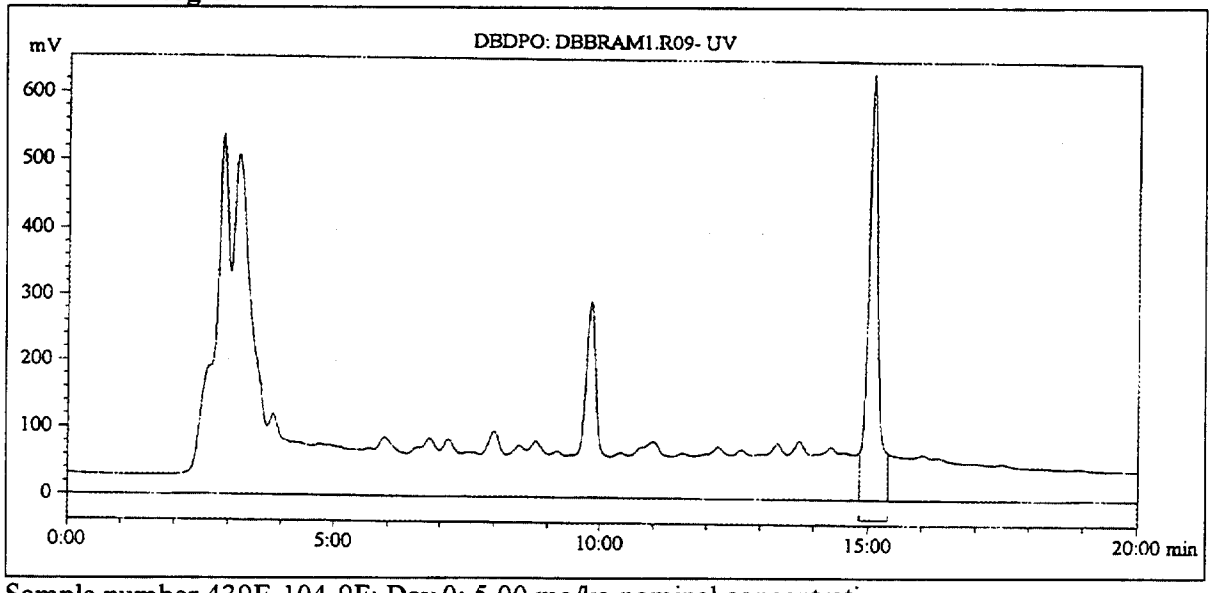
Chromatographic Profile of a Day 0 Test Sediment

Radiochromatogram



Sample number 439E-104-9F; Day 0, 5.00 mg/kg nominal concentration

UV Chromatogram

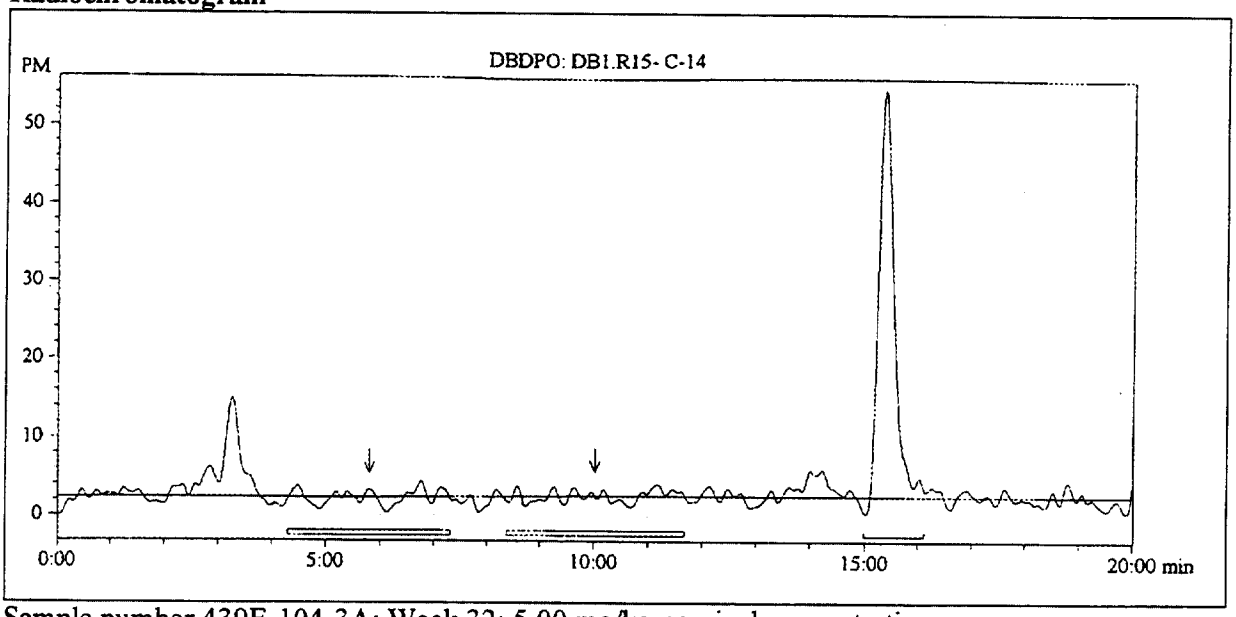


Sample number 439E-104-9F; Day 0; 5.00 mg/kg nominal concentration

Figure 11

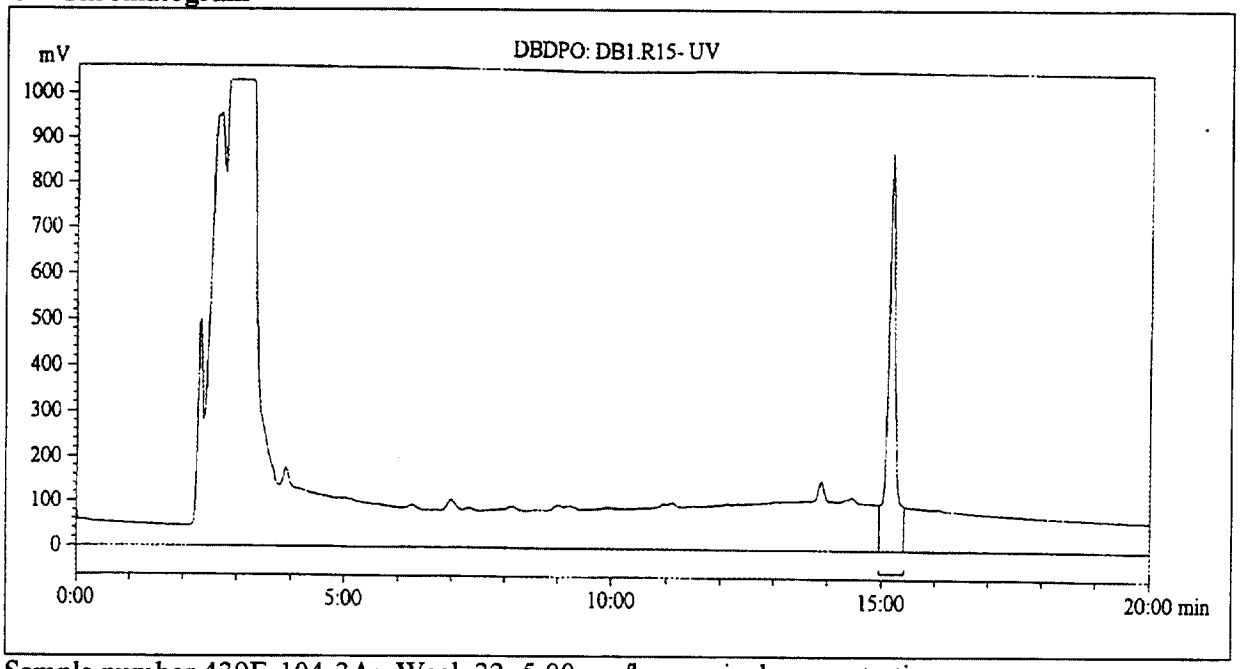
Chromatographic Profile of a Week-32 Test Sediment

Radiochromatogram



Sample number 439E-104-3A; Week 32; 5.00 mg/kg nominal concentration

UV Chromatogram

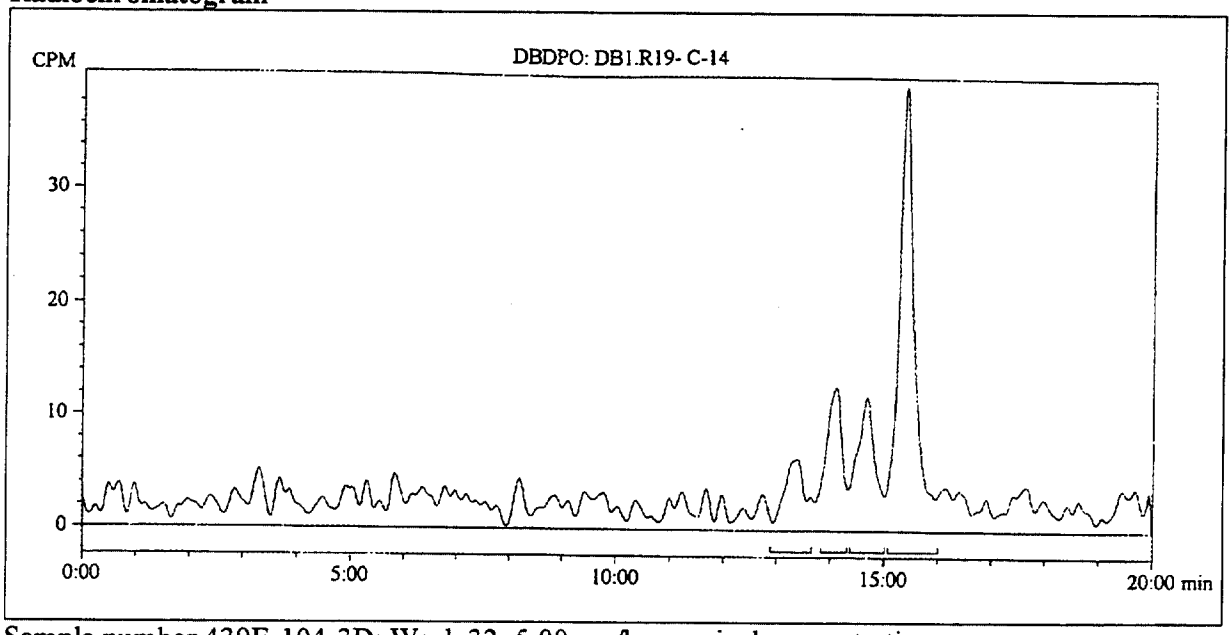


Sample number 439E-104-3A; Week 32; 5.00 mg/kg nominal concentration

Figure 12

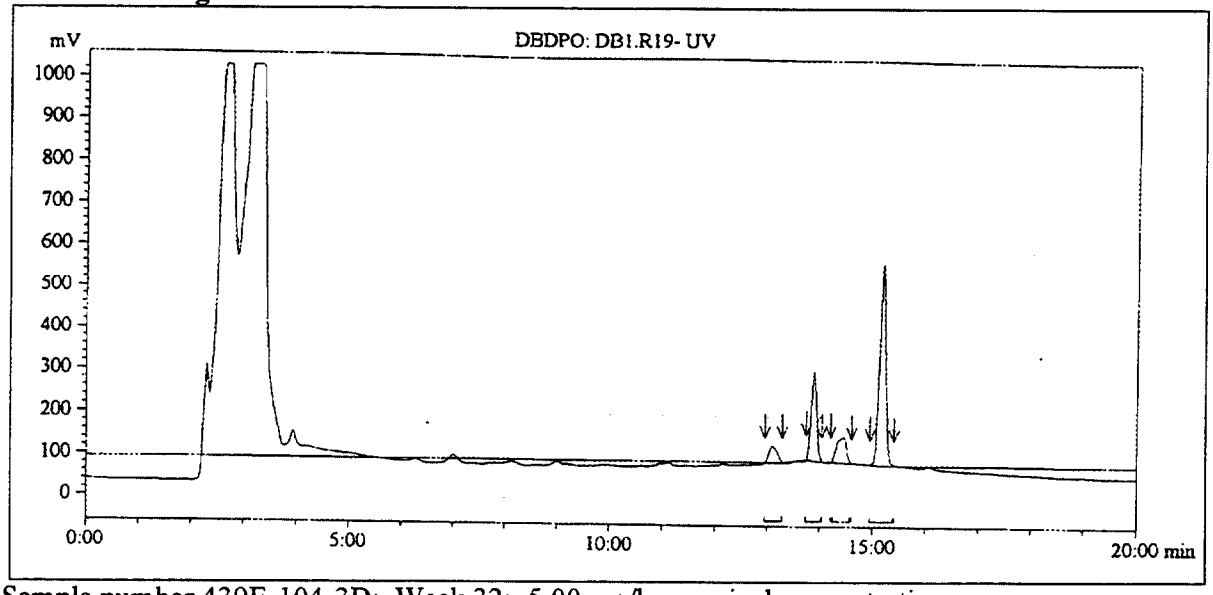
Chromatographic Profile of Test Sediment Containing Radiolabelled Components Eluting Prior to DBDPO

Radiochromatogram



Sample number 439E-104-3D; Week 32; 5.00 mg/kg nominal concentration

UV Chromatogram



Sample number 439E-104-3D; Week 32; 5.00 mg/kg nominal concentration

Appendix 1

Mean Cumulative Evolution of $^{14}CO_2$ and $^{14}CH_4$ (% of dosed ^{14}C) for Radiolabelled [^{14}C]-d-Glucose In Freshwater Sediment at 5 mg/kg[1, 2]

Day	Cumulative % $^{14}CO_2$ Gas Evolved (Mean ± Std. Dev.)	Cumulative % $^{14}CH_4$ Gas Evolved (Mean ± Std. Dev.)	Cumulative % Total ^{14}C Gas Evolved (Mean ± Std. Dev.)
5	36.61 ± 4.78	5.46 ± 1.89	42.07 ± 6.67
11	44.25 ± 3.45	9.63 ± 0.74	53.88 ± 2.72
18	49.07 ± 2.52	12.52 ± 2.54	61.58 ± 0.01
25	50.90 ± 0.29	13.92 ± 2.96	64.81 ± 2.67
32	52.16 ± 1.29	14.71 ± 2.91	66.87 ± 4.21
39	53.57 ± 2.10	15.33 ± 2.81	68.90 ± 4.91
46	55.09 ± 2.14	15.78 ± 2.63	70.87 ± 4.77
53	56.28 ± 2.12	16.13 ± 2.47	72.41 ± 4.59
60	57.35 ± 2.09	16.42 ± 2.28	73.76 ± 4.37
67	58.28 ± 2.04	16.64 ± 2.11	74.92 ± 4.15
74	59.12 ± 2.12	16.84 ± 1.97	75.96 ± 4.09
81	59.92 ± 2.21	17.01 ± 1.84	76.93 ± 4.04
88	60.63 ± 2.27	17.17 ± 1.68	77.80 ± 3.95
95	61.25 ± 2.31	17.30 ± 1.57	78.55 ± 3.88
102	61.85 ± 2.36	17.41 ± 1.48	79.26 ± 3.85
110	62.43 ± 2.38	17.51 ± 1.39	79.94 ± 3.77
117	62.67 ± 2.68	17.60 ± 1.31	80.26 ± 3.99
124	62.92 ± 2.93	17.74 ± 1.15	80.65 ± 4.09
131	63.30 ± 2.97	17.80 ± 1.09	81.10 ± 4.06
138	63.71 ± 2.98	17.86 ± 1.05	81.57 ± 4.03
145	64.03 ± 3.01	17.90 ± 1.03	81.93 ± 4.04
152	64.33 ± 3.07	17.93 ± 1.01	82.26 ± 4.08
159	64.63 ± 3.13	17.95 ± 1.00	82.58 ± 4.13
166	64.87 ± 3.15	17.98 ± 0.98	82.84 ± 4.13
173	65.11 ± 3.17	18.01 ± 0.99	83.12 ± 4.16
180	65.36 ± 3.18	18.06 ± 1.00	83.42 ± 4.18
188	65.57 ± 3.22	18.08 ± 1.00	83.65 ± 4.23
194	65.73 ± 3.25	18.10 ± 1.01	83.82 ± 4.26
201	65.89 ± 3.26	18.11 ± 1.02	84.00 ± 4.28
208	66.03 ± 3.29	18.12 ± 1.03	84.14 ± 4.31
215	66.15 ± 3.30	18.12 ± 1.03	84.26 ± 4.33
222	66.27 ± 3.32	18.12 ± 1.03	84.38 ± 4.34
226	67.20 ± 2.11	18.14 ± 1.05	85.33 ± 3.17

[1]Calculations were performed using Excel 2000. Small variances may exist for certain percentage values displayed in the table as a result of rounding of significant figures.

[2]Values represent the mean (± standard deviation) of two replicate test chambers.

Appendix 1 (Continued)

Mean Cumulative Evolution of $^{14}CO_2$ and $^{14}CH_4$ (% of dosed ^{14}C) for Radiolabelled [^{14}C]-DBDPO In Freshwater Sediment at 5 mg/kg[1, 2]

Day	Cumulative % $^{14}CO_2$ Gas Evolved (Mean ± Std. Dev.)	Cumulative % $^{14}CH_4$ Gas Evolved (Mean ± Std. Dev.)	Cumulative % Total ^{14}C Gas Evolved (Mean ± Std. Dev.)
5	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.01
11	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.01
18	0.01 ± 0.02	0.01 ± 0.01	0.03 ± 0.01
25	0.06 ± 0.03	0.05 ± 0.01	0.10 ± 0.03
32	0.10 ± 0.03	0.08 ± 0.01	0.18 ± 0.03
39	0.14 ± 0.02	0.12 ± 0.02	0.26 ± 0.03
46	0.18 ± 0.02	0.17 ± 0.02	0.35 ± 0.03
53	0.22 ± 0.02	0.20 ± 0.02	0.42 ± 0.03
60	0.22 ± 0.03	0.20 ± 0.02	0.43 ± 0.03
67	0.23 ± 0.03	0.21 ± 0.02	0.43 ± 0.03
74	0.23 ± 0.04	0.21 ± 0.02	0.44 ± 0.04
81	0.23 ± 0.04	0.22 ± 0.02	0.45 ± 0.04
88	0.25 ± 0.04	0.22 ± 0.02	0.47 ± 0.05
95	0.26 ± 0.04	0.22 ± 0.02	0.48 ± 0.05
102	0.27 ± 0.04	0.23 ± 0.02	0.50 ± 0.06
110	0.28 ± 0.04	0.23 ± 0.02	0.51 ± 0.05
117	0.28 ± 0.03	0.24 ± 0.02	0.52 ± 0.04
124	0.29 ± 0.04	0.24 ± 0.02	0.53 ± 0.05
131	0.29 ± 0.04	0.24 ± 0.02	0.53 ± 0.05
138	0.30 ± 0.04	0.26 ± 0.02	0.56 ± 0.05
145	0.30 ± 0.04	0.27 ± 0.02	0.57 ± 0.06
152	0.32 ± 0.04	0.27 ± 0.03	0.59 ± 0.06
159	0.32 ± 0.04	0.29 ± 0.03	0.61 ± 0.07
166	0.34 ± 0.04	0.30 ± 0.03	0.63 ± 0.07
173	0.36 ± 0.05	0.32 ± 0.03	0.68 ± 0.07
180	0.40 ± 0.04	0.36 ± 0.03	0.76 ± 0.07
188	0.41 ± 0.04	0.38 ± 0.04	0.79 ± 0.06
194	0.42 ± 0.04	0.39 ± 0.04	0.81 ± 0.06
201	0.44 ± 0.04	0.40 ± 0.04	0.85 ± 0.06
208	0.44 ± 0.04	0.40 ± 0.04	0.85 ± 0.06
215	0.44 ± 0.04	0.40 ± 0.04	0.85 ± 0.06
222	0.44 ± 0.04	0.41 ± 0.04	0.85 ± 0.06
226	0.44 ± 0.04	0.41 ± 0.04	0.85 ± 0.06

[1]Calculations were performed using Excel 2000. Small variances may exist for certain percentage values displayed in the table as a result of rounding of significant figures.

[2]Values represent the mean (± standard deviation) of three replicate test chambers.

Appendix 1 (Continued)

Mean Cumulative Evolution of $^{14}CO_2$ and $^{14}CH_4$ (% of dosed ^{14}C) for Radiolabelled [^{14}C]-DBDPO In Freshwater Sediment at 500 mg/kg[1, 2]

Day	Cumulative % $^{14}CO_2$ Gas Evolved (Mean ± Std. Dev.)	Cumulative % $^{14}CH_4$ Gas Evolved (Mean ± Std. Dev.)	Cumulative % Total ^{14}C Gas Evolved (Mean ± Std. Dev.)
5	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
11	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02
18	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02
25	0.06 ± 0.02	0.06 ± 0.01	0.12 ± 0.02
32	0.10 ± 0.01	0.10 ± 0.01	0.20 ± 0.01
39	0.14 ± 0.01	0.15 ± 0.02	0.28 ± 0.02
46	0.18 ± 0.01	0.19 ± 0.02	0.37 ± 0.03
53	0.21 ± 0.01	0.23 ± 0.03	0.43 ± 0.03
60	0.21 ± 0.01	0.23 ± 0.02	0.44 ± 0.03
67	0.21 ± 0.01	0.23 ± 0.02	0.44 ± 0.03
74	0.22 ± 0.01	0.23 ± 0.02	0.45 ± 0.03
81	0.22 ± 0.01	0.23 ± 0.02	0.45 ± 0.03
88	0.23 ± 0.01	0.24 ± 0.02	0.47 ± 0.03
95	0.23 ± 0.02	0.24 ± 0.01	0.47 ± 0.03
102	0.24 ± 0.01	0.24 ± 0.02	0.48 ± 0.03
110	0.24 ± 0.01	0.24 ± 0.02	0.48 ± 0.03
117	0.24 ± 0.01	0.25 ± 0.02	0.48 ± 0.03
124	0.24 ± 0.01	0.26 ± 0.01	0.50 ± 0.02
131	0.24 ± 0.01	0.27 ± 0.02	0.51 ± 0.03
138	0.26 ± 0.02	0.28 ± 0.01	0.54 ± 0.03
145	0.26 ± 0.02	0.29 ± 0.02	0.55 ± 0.04
152	0.27 ± 0.02	0.29 ± 0.02	0.57 ± 0.04
159	0.29 ± 0.02	0.30 ± 0.02	0.60 ± 0.04
166	0.30 ± 0.02	0.32 ± 0.05	0.62 ± 0.06
173	0.33 ± 0.02	0.35 ± 0.05	0.67 ± 0.07
180	0.36 ± 0.02	0.38 ± 0.06	0.74 ± 0.07
188	0.38 ± 0.03	0.39 ± 0.07	0.77 ± 0.09
194	0.39 ± 0.03	0.39 ± 0.06	0.78 ± 0.08
201	0.39 ± 0.03	0.40 ± 0.06	0.79 ± 0.07
208	0.39 ± 0.03	0.40 ± 0.06	0.79 ± 0.07
215	0.39 ± 0.03	0.40 ± 0.06	0.79 ± 0.07
222	0.39 ± 0.03	0.40 ± 0.06	0.79 ± 0.07
226	0.39 ± 0.03	0.40 ± 0.06	0.79 ± 0.07

[1]Calculations were performed using Excel 2000. Small variances may exist for certain percentage values displayed in the table as a result of rounding of significant figures.

[2]Values represent the mean (± standard deviation) of three replicate test chambers.

Appendix 2

Quality Control Samples of DBDPO in Sediment by HPLC/UV Detection

Sample		Concentration (mg/L)		Percent Recovery[2]
Number (439E-104-)	Type	Fortified	Measured [2]	
MAB-3	Matrix Blank	0.0	< LOQ[1]	--
MAB-4	Matrix Blank	0.0	< LOQ	--
MAB-5	Matrix Blank	0.0	< LOQ	--
MAB-6	Matrix Blank	0.0	< LOQ	--
MAB-7	Matrix Blank	0.0	< LOQ	--
MAB-8	Matrix Blank	0.0	< LOQ	--
MAB-9	Matrix Blank	0.0	< LOQ	--
MAS-5	Matrix Fortification	5.00	5.09	102
MAS-6	Matrix Fortification	500	493	98.7
MAS-7	Matrix Fortification	5.00	5.04	101
MAS-8	Matrix Fortification	500	461	92.2
MAS-9	Matrix Fortification	5.00	5.41	108
MAS-10	Matrix Fortification	500	431	86.1
MAS-11	Matrix Fortification	5.00	3.72	74.5
MAS-12	Matrix Fortification	500	417	83.4
MAS-13	Matrix Fortification	5.00	4.54	90.8
MAS-14	Matrix Fortification	500	481	96.2
MAS-15	Matrix Fortification	5.00	2.91	58.1
MAS-16	Matrix Fortification	500	412	82.4
MAS-17	Matrix Fortification	5.00	4.85	97.0
MAS-18	Matrix Fortification	5.00	4.92	98.4

[1] The limit of quantitation (LOQ) was 1.25 µg/g, calculated as the product of the lowest calibration standard (0.0500 mg/L) and the dilution factor of the matrix blank samples (25.0).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

Appendix 3

Summary of Analytical Chemistry Data for DBDPO in Sediment by HPLC/UV Detection

Week 32

Nominal Test Concentration (µg/g)	Sample Number (439E-104-)	Measured Concentration (µg/g)[2]	Percent of Nominal[2]	Mean Measured Concentration (µg/g)	Mean Measured Percent of Nominal
0.0 (Control)	1A (1st)	< LOQ[1]	--	< LOQ	--
	1A (2nd)	< LOQ	--		
	1B	< LOQ	--		
	1C	< LOQ	--		
	1D	< LOQ	--		
	1E	< LOQ	--		
	1F	< LOQ	--		
	1G	< LOQ	--		
5.00	2A (1st)	9.19	184	9.20	184
	2A (2nd)	9.29	186		
	2B	9.26	185		
	2C	9.17	183		
	2D	9.24	185		
	2E	9.15	183		
	2F	9.04	181		
	2G	9.28	186		
5.00	3A (1st)	5.91	118	6.16	123
	3A (2nd)	5.89	118		
	3B	6.68	134		
	3C	6.25	125		
	3D	6.10	122		
	3E	5.89	118		
	3F	5.92	118		
	3G	6.67	133		
5.00	4A (1st)	4.15	83.0	4.13	82.6
	4A (2nd)	4.15	83.0		
	4B	4.08	81.5		
	4C	4.60	91.9		
	4D	3.83	76.6		
	4E	4.73	94.6		
	4F	3.78	75.7		
	4G	3.73	74.6		

[1] The limit of quantitation (LOQ) was 1.25 µg/g, calculated as the product of the lowest calibration standard (0.0500 mg/L) and the dilution factor of the matrix blank samples (25.0).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

NOTE: Duplicate injections were performed for the first sample of each set.

Appendix 3 (Continued)

Summary of Analytical Chemistry Data for DBDPO in Sediment by HPLC/UV Detection

Week 32

Nominal Test Concentration (µg/g)	Sample Number (439E-104-)	Measured Concentration (µg/g)[2]	Percent of Nominal[2]	Mean Measured Concentration (µg/g)	Mean Measured Percent of Nominal
0.0 (control)	5A (1st)	< LOQ[1]	--	< LOQ	--
	5A (2nd)	< LOQ	--		
	5B	< LOQ	--		
	5C	< LOQ	--		
	5D	< LOQ	--		
	5E	< LOQ	--		
	5F	< LOQ	--		
	5G	< LOQ	--		
500	6A (1st)	839	168	768	154
	6A (2nd)	840	168		
	6B	809	162		
	6C	673	135		
	6D	720	144		
	6E	725	145		
	6F	761	152		
	6G	776	155		
500	7A (1st)	703	141	670	134
	7A (2nd)	703	141		
	7B	693	139		
	7C	569	114		
	7D	724	145		
	7E	637	127		
	7F	639	128		
	7G	694	139		
500	8A (1st)	444	88.9	417	83.4
	8A (2nd)	444	88.9		
	8B	337	67.5		
	8C	454	90.8		
	8D	440	88.0		
	8E	363	72.7		
	8F	403	80.6		
	8G	447	89.5		

[1] The limit of quantitation (LOQ) was 1.25 µg/g, calculated as the product of the lowest calibration standard (0.0500 mg/L) and the dilution factor of the matrix blank samples (25.0).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

NOTE: Duplicate injections were performed for the first sample of each set.

Appendix 3 (Continued)

Summary of Analytical Chemistry Data for DBDPO in Sediment by HPLC/UV Detection

Day 0

Nominal Test Concentration (µg/g)	Sample Number (439E-104-)	Measured Concentration (µg/g)[2]	Percent of Nominal[2]	Mean Measured Concentration (µg/g)	Mean Measured Percent of Nominal
5.00	9A (1st)	6.17	123	6.02	120
	9A (2nd)	6.17	123		
	9B	5.88	118		
	9C	6.08	122		
	9D	6.15	123		
	9E	6.08	122		
	9F	5.57	111		
	9G	6.07	121		
5.00	10A (1st)	7.48	150	7.30	146
	10A (2nd)	7.57	151		
	10B	7.30	146		
	10C	7.40	148		
	10D	6.75	135		
	10E	7.04	141		
	10F	7.51	150		
	10G	7.37	147		
500	15A (1st)	541	108	492	98.4
	15A (2nd)	538	108		
	15B	382	76.5		
	15C	538	108		
	15D	502	100		
	15E	537	107		
	15F	499	100		
	15G	397	79.4		
500	16A (1st)	608	122	602	120
	16A (2nd)	608	122		
	16B	599	120		
	16C	616	123		
	16D	588	118		
	16E	567	113		
	16F	602	120		
	16G	625	125		

[1] The limit of quantitation (LOQ) was 1.25 µg/g, calculated as the product of the lowest calibration standard (0.0500 mg/L) and the dilution factor of the matrix blank samples (25.0).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

NOTE: Duplicate injections were performed for the first sample of each set.

Appendix 4

Sediment and Characterization Reports

AGVISE LABORATORIES

Highway 15
P.O. Box 510
Northwood, ND 58267
(701) 587-6010
FAX (701) 587-6013
email: agvise@polarcomm.com
Homepage: agviselabs.com

AGVISE Soil Characterization Report

Submitting firm = WILDLIFE INTERNATIONAL
Protocol or Study No = 439E-104
Sample ID. = FRESHWATER SEDIMENT
Trial ID. = NA
Date Received = 3-13-00
Date Reported = 03-20-2000

AGVISE Lab No	00- 62
Percent Sand	50
Percent Silt	29
Percent Clay	21
USDA Textural Class (hydrometer method)	Loam
Bulk Density (disturbed) gm/cc	1.00
Cation Exchange Capacity (meq/100 g)	8.6
% Moisture at 1/3 Bar	27.6
Percent Organic Matter	1.4
pH in 1:1 soil:water ratio	6.3

Base Saturation Data

Cation	Percent	ppm
Calcium	46.4	800
Magnesium	21.2	220
Sodium	2.7	54
Potassium	1.9	63
Hydrogen	27.8	24

These tests were completed in compliance of 40 CFR Part 160.

Robert Deutsch — 3-20-00
Soil Scientist/Analytical Investigator — Date

METHOD SUMMARY FOR SOIL ANALYSIS

TESTING LABORATORY: **AGVISE LABORATORIES, INC.**
P.O. BOX 510; Highway 15
Northwood, ND 58267
(701)-587-6010

The following is a summary of analytical methods used by AGVISE Laboratories in the determination of soil characteristics and nutrient content. Analytical data of some or all of these analytical methods are presented based upon the testing requested by the firm submitting the soil specimens.

Chemical Properties

Carbonates-Determined by gravimetric loss of carbon dioxide (NUT.02.14).

Cation Exchange Capacity - Determined by summing the cations with hydrogen (NUT.02.03). The cations of Magnesium, Potassium, Calcium, and Sodium are determined by extraction with 1.0 N ammonium acetate (NUT.02.12). Hydrogen is determined by measuring the pH of the soil in Adams-Evans Buffer Solution (NUT.02.11).

Nitrogen, % Total - Determined by the Kjeldahl method (NUT.02.15).

Organic Carbon % - Determined by the Walkley-Black procedure (NUT.02.20).

Organic Matter % - Determined by the Walkley-Black Procedure (NUT.02.09) in soils with less than 10% organic matter. Determined by the loss of weight on ignition procedure (NUT.02.04) in soils with a 10% or more organic matter.

pH - Determined with a pH electrode in a 1:1 soil:water suspension (NUT.02.05) except when specified by state regulations to use a saturated paste (NUT.02.39).

Phosphorus - Determined by the Olsen method (NUT.02.07).

Soluble Salts - Determined using a conductivity meter in a 1:1 soil:water suspension (NUT.02.19).

Physical Properties

% Gravel - Determined by dry sieving and weighing the fraction over 2 mm (NUT.02.16).

% Sand, Silt, and Clay - Determined by hydrometer method (NUT.02.06) or by pipette method (NUT.02.56).

Sand Particle Size - Determined by weighing fractions obtained by wet sieving (NUT.02.32).

AGVISE LABORATORIES

Highway 15
P.O. Box 510
Northwood, ND 58267
(701) 587-6010
FAX (701) 587-6013
email: agvise@polarcomm.com
Homepage: agviselabs.com

The following personnel have been duly trained to perform Plant Analysis, Soil and Water Characterization methods under 40 CFR Part 160 Good Laboratory Practice Standards.

Technical Support Staff

Anderson, Linda M. - Technician III
Deutsch, Robert L. - President
Hart, Linda M. - Technician III
Hime, Sherry L. - Technician I
Johnson, Julie M. - Nutrient Laboratory Manager
McNeil, Vigo (Art) - Technician I
Moen, Lucinda S. - Technician III
Pollert, Garis - Nutrient Laboratory Analyst
Wall, Mary J. - Technician I
Wyant, Linda L. - Technician I

Office Support Staff

Berg, Eileen A. - Secretary III
Ducioame, Gail M. - Quality Control Specialist
Fuglestad, Teresa S. - Secretary II
Hagen, Shelly J. - Administrative Assistant

Quality Assurance

Thingelstad, Mary L. - Quality Assurance Manager

10/99

AGVISE LABORATORIES

Highway 15
P.O. Box 510
Northwood, ND 58267
(701) 587-6010
FAX (701) 587-6013
email: agvise@polarcomm.com
Homepage: agviselabs.com

AGVISE Water Characterization Report

Submitting firm:	= WILDLIFE INTERNATIONAL LTD.
Protocol or Study No	= 439E-104
Sample ID.	= SURFACE WATER
Trial ID.	= NA
Date Received	= 3-13-00
Date Reported	= 3-16-00

AGVISE Lab No	00-0052	
pH		7.6
Total Phosphorus		0.6 ppm
Sulfate-Sulfur		44 ppm
Nitrate-Nitrogen		2.7 ppm

These tests were completed in compliance of 40 CFR Part 160.

Robert Deutsch
Soil Scientist/Analytical Investigator

3-17-00
Date

METHOD SUMMARY FOR WATER ANALYSIS

TESTING LABORATORY: **AGVISE LABORATORIES, INC.**
P.O. BOX 510; Highway 15
Northwood, ND 58267
(701)-587-6010

The following is a summary of analytical methods used by AGVISE Laboratories in the determination of water characteristics and nutrient content. Analytical data of some or all of these analytical methods are presented based upon the testing requested by the firm submitting the water specimens.

Alkalinity - Determined by titration with 1N sulfuric acid (NUT.02.03).

Carbonate and Bicarbonate - Determined by titration using 1N sulfuric acid and 0.25% phenophthalieum in 50% ethanol (NUT.02.26).

Cations Ca, Na and Mg - The cations are determined by atomic absorption spectrophotometry (NUT.02.23).

Chemical Oxygen Demand - Chemical Oxygen Demand is determined by measuring the portion of the organic matter susceptible to oxidation by a strong oxidant (NUT.02.38).

Conductivity - Determined by using a conductivity meter (NUT.02.22).

Hardness - Calculated from the Ca & Mg content in a water specimen (NUT.02.18).

Nitrogen, Total - Determined by the Kjeldahl procedure (NUT.02.28).

Organic Matter - Determined by comparing an ashed sample (550°C) with total dissolved solids (NUT.02.46).

Oxygen, Dissolved - Determined by using the Azide modification of the Winkler titration method (NUT.02.37).

pH - Determined by using a pH electrode (NUT.02.17).

Redox Potential - Redox Potential is measured using a platinum Redox electrode (NUT.02.45).

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Berg, Eileen A. - Secretary III
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Fuglestad, Teresa S. - Secretary II
Hagen, Shelly J. - Administrative Assistant

Quality Assurance

Thingelstad, Mary L. - Quality Assurance Manager

COPY OF ORIGINAL
AGVISE Laboratories, Inc.
Initial [illegible] Date 3-7-00

10/99

Appendix 5

Protocol, Amendments and Deviation

PROTOCOL

POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

Submitted to

Chemical Manufacturers Association's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

November 10, 1999

POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED
DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

SPONSOR:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Edward C. Schaefer
LABORATORY MANAGEMENT:	Henry O. Krueger, Ph.D. Director of Aquatic Toxicology & Non-Target Plants

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: 2/2/00 Experimental Termination Date: 9/13/00

Project No.: 439E-104 Study Room: 45

Test Concentrations: 5 & 500 mg/Kg

Test Substance No.: 5160/3578 Reference Substance No. (if applicable): 4771

PROTOCOL APPROVAL

Edward C. Schaefer
STUDY DIRECTOR — DATE 1/20/2000

H. Krueger
LABORATORY MANAGEMENT — DATE 1/20/00

Wendy K. Sherman
SPONSOR'S REPRESENTATIVE — DATE 11/11/99

OBJECTIVE

The objective of the study is to determine the rate and extent of biotransformation of a nonvolatile radiolabelled test material under anaerobic conditions in a flooded sediment. Anaerobic sediment will be dosed with ^{14}C-labelled decabromodiphenyl oxide (DBDPO) and incubated under anaerobic conditions. Evolved $^{14}CO_2$ and $^{14}CH_4$ will be trapped continuously using a trapping/combustion train and quantified by liquid scintillation counting (LSC). The total amount of radioactivity recovered as $^{14}CO_2$ and $^{14}CH_4$ will be expressed as a percent of the amount of radioactivity dosed. Sediment will be analyzed for the test material and metabolites.

EXPERIMENTAL DESIGN

The test will contain one reference and two treatment groups that will be used to monitor the production of $^{14}CO_2$ and $^{14}CH_4$. The reference group will contain two replicate test chambers and will be dosed with a combination of unlabelled and ^{14}C-labelled glucose at a concentration of 5 mg/Kg. The two treatment groups will contain 3 replicate test chambers and will be used to evaluate the biotransformation of the test substance at 5 and 500 mg/Kg. The test chambers will be incubated at ambient room temperature and the production of $^{14}CO_2$ and $^{14}CH_4$ will be monitored over a period of 32 weeks. The headspace of the test chambers will be continuously purged with nitrogen and then passed through two CO_2 traps. The effluent gas from the CO_2 traps will be channeled through a quartz column packed with cupric oxide at 800°C in a tube furnace to combust methane to CO_2. The gas exiting the combustion column will be passed through two additional CO_2 traps. CO_2 traps will be periodically collected and analyzed for radioactivity by liquid scintillation counting (LSC). A the end of the 32- week test period, samples from each of the reference and treatment group test chambers will be analyzed for DBDPO and metabolites (if any). The results from the reference sediments will be used to provide information about the contamination of the sediment prior to the start of the test.

Six additional treatment chambers will be prepared at both 5 and 500 mg/Kg but will not be attached to the headspace gas collection system. Samples from the additional test chambers will be analyzed for DBDPO and metabolites (if any) only if significant degradation of DBDPO is observed at the end of the 32 -week test period. Analysis of the additional samples will be initiated by protocol amendment.

MATERIALS AND METHODS

Test methods are based on the procedures described by Nuck and Federle (1).

Test Substance

Information on the characterization of test, control or reference substances is required by OECD Principles of Good Laboratory Practice. The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test substance has been characterized according to GLPs. If written verification of GLP test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report. The attached form **IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR** (Appendix II) is to be used to provide information necessary for GLP compliance.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Test Substance Preparation

Using the radiolabelled form (and unlabeled form as needed), a dosing material will be prepared at an active concentration that facilitates the addition of the test substance to the test chambers. The activity of the dosing material will be measured by combustion, and the radioactivity added to each test chamber should be ≥ 1μCi. The test substance will be administered by direct weight addition. Direct weight addition is the most appropriate route of administration.

Reference Substance Preparation

Using the radiolabelled and unlabeled forms of d-glucose, a dosing solution will be prepared in NANO®pure water at a concentration that facilitates the addition of the test substance to the reference chambers. The activity of the dosing material will be measured by LSC, and the radioactivity added to each test chamber should be ≥ 1μCi. The reference substance will be administered by volumetric addition.

Test Inoculum

Sediment and accompanying surface water will be collected from the Schuykill river, Valley Forge, Pennsylvania. Upon collection, the redox potential of the sediment will be measured. The percent moisture of the sediment will be measured. Sediment may be stored at room temperature in an anaerobic chamber for

up to 7 days. Prior to use, the surface water will be decanted from above the sediment and placed in a separate container. The surface water and sediment will be characterized by Agvise Laboratories, Inc. (Northwood, North Dakota). The sediment characterization will include pH, % organic matter (Walkey Black), cation exchange capacity (Ca, Mg, Na, K & H), disturbed bulk density, % sand-silt-clay, USDA textural class, and water holding capacity (1/3 bar). The surface water characterization will include pH, nitrate-nitrogen, sulfate-sulfur, and total phosphorus. A 2 mg resazurin/L solution will be prepared using the surface water.

Test Apparatus and Conditions

The test chambers will be graduated 500 mL glass bottles and will be identified by project number, test substance ID, test concentration, and unique identifier. The headspace gases within each of the test chambers will be continuously purged with a flow of nitrogen (approximately 5 mL/min.) and passed through a gas collection system consisting of two sets of CO_2 traps and a combustion apparatus. The displaced gases will initially pass through one empty bottle followed by two bottles each containing 100 mL of 1.5N KOH (CO_2 trapping solution) followed by another empty bottle. The gas will be combined with a flow of oxygen (approximately 2 mL/min) and channeled through a quartz column that is packed with cupric oxide and maintained at approximately 800°C in a tube furnace to combust methane to CO_2. The gas exiting the combustion column will be passed through an empty bottle followed by two additional CO_2 traps. The test chambers will be incubated in a water bath at room temperature. Water temperatures will be measured each working day.

Preparation of the Test Chambers

The test chambers will be transferred to an anaerobic chamber. Sufficient sediment to reach the 300 mL graduation will be added to each chamber. The test chambers will be allowed to equilibrate overnight. After the equilibration period, the appropriate amounts of test or reference substance will be added to their respective test chamber. The sediment systems will be mixed using a wooden applicator so that the test and substances are distributed throughout the top 1 inch of sediment. The numbers of bacteria are typically highest in surface sediments and decrease rapidly within sediments at greater depths (2). The lower part of the wooden applicator will be broken off and left in the test chamber. Approximately 10 mL of the resazurin/surface water solution will be added to each chamber. The chambers then will be sealed and transferred out of the anaerobic chamber and connected to the gas collection system. The additional test

chambers that are not connected to the gas collection system will be stoppered with a gas trap and incubated at approximately 22°C within the anaerobic chamber.

Sample Collection and Analysis

The 1st CO_2 trap of each set (before and after combustion apparatus) will be removed once a week over the test periods. Three replicate 1 mL aliquots of each trap will be analyzed for radioactivity by LSC. More or less frequent sampling may be conducted at the discretion of the Study Director. The 2nd trap in each set will be moved to the 1st position and a new trap will be placed in the 2nd trap spot.

Two chambers from 5 and 500 mg/Kg treatments that were prepared but will not be attached to the headspace gas collection system will be acidified using 10 mL of concentrated sulfuric acid on Day 0 and weeks 13 and 26. Acidified test chambers will be stored in a refrigerator until analysis (if any). Samples from the additional test chambers will be analyzed for DBDPO and metabolites (if any) if requested by the Sponsor's Representative. Analysis of the additional samples will be initiated by protocol amendment.

Test Termination

At the end of the 32 -week test period, the contents of the test chambers will be acidified by the addition of 10 mL of concentrated sulfuric acid. The chambers will be purged for approximately 24 hours. After purging the pH of the sediment will be measured. If the measured pH is >2.0, an additional 10 mL of concentrated sulfuric acid will be added to the chambers and the chambers will be purged for approximately 24 hours. If the measured pH is < 2.0, the remaining traps will be analyzed by LSC.

The contents of the reference and treatment group test chambers will be air dried at room temperature and then homogenized using a motar and pestle. Aliqouts of the dried sediments will be analyzed for DBDPO and metabolites (if any). The chemical analysis of the samples will be performed by Wildlife International, Ltd. The analytical methods used will be based upon methodology developed in consultation with the Sponsor and will be amended to the protocol.

Mass Balance Determination

After the test has been terminated and the CO_2 traps have been removed for analysis, the mass balance determination will be performed. Three replicate samples of the dried sediments will be combusted using a Packard oxidizer (or equivalent).

Calculations

The amount of $^{14}CO_2$ & $^{14}CH_4$ evolved will be calculated using the following equations (A&B):

A) $\frac{(CO_2\ dpm \times 100)}{initial\ dpms} = \%\ radioactivity\ recovered\ as\ CO_2$

B) $\frac{(CH_4\ dpm \times 100)}{initial\ dpms} = \%\ radioactivity\ recovered\ as\ CH_4$

where:

Initial radioactivity = total dpms added to test chamber, and

CO_2 (or CH_4) dpms = mean of replicates of 1 mL trapping solution

The radioactivity associated with the sediment will be calculated using the following equation (C):

C) $\frac{sediment\ dpms}{initial\ dpms} \times 100 = \%\ radioactivity\ remaining\ on\ sediment$

where:

mean of replicate 1 gram (dry weight) samples = solids dpms

A total mass balance will be calculated using the following equation:

Total Mass Balance = A + B + C

Treatment of Results

No bias is expected in this study. Statistics beyond the calculation of standard deviations and means will not be used in the evaluation of the results.

RECORDS TO BE MAINTAINED

Records to be maintained will include, but not limited to, the following:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance as provided by Sponsor.
3. Study initiation and termination dates.
4. Experimental initiation and termination dates.
5. Test substance concentration calculations and solution preparation.
6. Inoculum source and pretreatment data.
7. Results of LSC analysis.
8. Temperature range recorded during test period.
9. Copy of final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report is to include, but is not limited to, the following, when applicable:

1. Name and address of facility performing the study.
2. Dates on which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
5. Identification and characterization of the test substance as provided by Sponsor including name, CAS number, percent active, and other characteristics, if provided by the Sponsor.
6. A description of the transformations and calculations performed on the data.
7. Results of the LSC analysis performed.
8. A description of the test system.
9. A description of the preparation of the test solutions, the testing concentrations, the route of administration, and the duration of the test.
10. A description of all circumstances that may have affected the quality or integrity of the data.
11. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
12. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
13. The location where the raw data and final report are to be stored.

14. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and findings reported to the Study Director and Management.
15. Full description of analytical methods used in the study.

CHANGES TO THE FINAL REPORT

If it is necessary to make corrections or additions to the final report after it has been accepted, such changes shall be made in the form of an amendment issued by the Study Director. The amendment shall clearly identify the part of the study that is being amended and the reasons for the alteration. Amendments shall be signed and dated by the Study Director and Laboratory QA.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). Each study conducted by Wildlife International Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

REFERENCES

1 Nuck, B.A., Federle, T.W. 1996. *A Batch Test for Assessing the Mineralization of ^{14}C-Radiolabeled Compounds under Realistic Anaerobic Conditions.* Environmental Science & Technology.

2 Wetzel, R. G. 1975. Limnology. P592-593. W.B. Saunders Company, Philadelphia, Pa.

APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): _____

Test Substance Sample Code or Batch Number: _____

Test Substance Purity (% Active Ingredient): _____ Expiration Date: _____

Solubility: Water: _____ Theoretical Carbon Content : _____

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? Yes ____ No _____

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd.

_____

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes ____ No _____
Other pertinent stability information:

_____

IV. Test Concentrations: Adjust test concentration to 100% a.i.
____ based upon the purity (%) given above.

Do not adjust test concentration to 100%
____ a.i. Test the material AS IS.

V. Toxicity Information:

Mammalian: Rat LD50 _____ Mouse LD50 _____

Aquatic: Invertebrate Toxicity (EC/LC50) _____

Fish Toxicity (LC50) _____

Other Toxicity Information (including findings of chronic and subchronic tests):

_____

VI. Classification of the Compound:

_____ Insecticide _____ Herbicide _____ Fungicide _____
_____ Microbial Agent_____ Economic Poison _____

Other: _____

WILDLIFE INTERNATIONAL LTD.

PROJECT NO.: 439E-104
Page 1 of 1

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 1

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: March 09, 2000

AMENDMENT: Test Substance Preparation, Page-4-

DELETE: Using the radiolabelled form (and unlabeled form as needed), a dosing material will be prepared at an active concentration that facilitates the addition of the test substance to the test chambers. The activity of the dosing material will be measured by combustion, and the radioactivity added to each test chamber should be ≥ 1µCi. The test substance will be administered by direct weight addition. Direct weight addition is the most appropriate route of administration.

INSERT: Using the radiolabelled form, a dosing solution will be prepared in tetrahydrofuran (THF) at an active concentration that facilitates the addition of the test substance to the test chambers. The activity of the dosing solution will be measured by LSC. The radiolabelled test substance will be administered by volumetric addition to dried sediment. The dried sediment containing the labelled test substance will sit overnight (to allow for the dissipation of the tetrahydrofuran solvent) before being added to the test chambers. The radioactivity added to each test chamber should be ≥ 1µCi. To assess the effects of the solvent on the test system, an equivalent volume of THF will be administered to dried sediment and handled in an identical manner before being added to each reference chamber. A sufficient quantity of the nonlabelled test substance will be administered to each test chamber by direct weight addition to achieve the desired test concentrations.

REASON: The properties of the radiolabelled test material necessitated an alternative method of administration.

Edward C. Dell-Jr 7/6/00
STUDY DIRECTOR DATE

LABORATORY MANAGEMENT 7/6/00
DATE

Wendy K. Shannon 9/22/00
SPONSOR'S REPRESENTATIVE DATE

GF 7/6/2000

PROJECT NO.: 439E-104
Page 1 of 1

WILDLIFE INTERNATIONAL LTD.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 2

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: June 08, 2000

AMENDMENT: Experimental Design, Page-3-

DELETE: Six additional treatment chambers will be prepared at both 5 and 500 mg/Kg but will not be attached to the headspace gas collection system. Samples from the additional test chambers will be analyzed for DBDPO and metabolites (if any) only if significant degradation of DBDPO is observed at the end of the 32 -week test period. Analysis of the additional samples will be initiated by protocol amendment.

INSERT: Six additional treatment chambers will be prepared at both 5 and 500 mg/Kg but will not be attached to the headspace gas collection system. Samples from additional test chambers will be analyzed for DBDPO and metabolites (if any). Analysis of additional samples will be initiated by protocol amendment.

REASON: Sponsor's Representative requested preliminary quantification of DBDPO in several of the additional test chambers prior to the end of the 32-week test period.

P. C. Lahm — STUDY DIRECTOR — 7-17-01 — DATE

J. K. — LABORATORY MANAGEMENT — 7/17/01 — DATE

Wendy K. Shuman — SPONSOR'S REPRESENTATIVE — 7/19/01 — DATE

QA KH 7-17-01

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 3

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: June 08, 2000

AMENDMENT: Sample Collection and Analysis, Page-6-

DELETE: Acidified test chambers will be stored in a refrigerator until analysis (if any). Samples from the additional test chambers will be analyzed for DBDPO and metabolites (if any) if requested by the Sponsor's Representative. Analysis of the additional samples will be initiated by protocol amendment.

INSERT: Acidified test chambers will be stored in a refrigerator until analysis. Samples from the additional test chambers acidified on Day 0 and Week 13 will be extracted and analyzed for DBDPO using high performance liquid chromatography (HPLC) with UV detection. The extraction and analytical methods to be used are identified in Appendix II. Samples from extracted Day 0 and Week 13 sediments will be combusted using a Packard oxidizer (or equivalent) to determine residual radioactivity (if any) and extraction efficiency. Analysis of additional samples will be initiated by protocol amendment.

REASON: Analyses requested by the Sponsor's Representative.

P.A. Lemieux — STUDY DIRECTOR — 7/17/01 — DATE

LABORATORY MANAGEMENT — 7/17/01 — DATE

Wendy K. Sherman — SPONSOR'S REPRESENTATIVE — 7/19/01 — DATE

QA KH 7-17-01

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 4

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: June 08, 2000

AMENDMENT: Appendix II, Page-12-

INSERT:

APPENDIX II

Method Outline for the Extraction and HPLC/UV Analysis of DBDPO in Sediment

1. Pre-rinse all glassware with tetrahydrofuran.
2. Prepare recovery samples by directly fortifying 10.0 g of sediment (contained in 8-oz French square bottles) with the appropriate DBDPO stock solution. Unfortified sediment will serve as the matrix blank. For test samples, weigh 10.0 g of each into 8-oz French square bottles.
3. To each recovery and study sample add 100 mL of tetrahydrofuran. Seal samples and place on a shaker table for ~15 minutes at a setting of 250 rpm.
4. Centrifuge samples ~ 5 minutes at a setting of 1500 rpm.
5. Pour the extracts through glass wool contained in glass funnels into roundbottom flasks.
6. Repeat the extraction procedure using an additional 100-mL of tetrahydrofuran and combine the extracts in their respective roundbottom flasks.
7. Rotary evaporate the samples to ~2-3 mL.
8. Quantitatively transfer the concentrated extract using tetrahydrofuran to the appropriate size volumetric flask.
9. Perform secondary dilutions where appropriate using 50% tetrahydrofuran: 50% water.
10. Filter aliquots from each extract through 0.45 acrodiscs directly into an autosampler vials and submit samples for HPLC/UV analysis.
11. Analyze with a CH_3CN:H_2O:H_3PO_4 gradient on a Zorbax phenyl column with detection at 220 nm.

Any changes to the method will be documented in the raw data and described in the final report.

REASON: Analysis requested by the Sponsor's Representative.

P.C. [signature] 7-17-01
STUDY DIRECTOR DATE

[signature] 7/17/01
LABORATORY MANAGEMENT DATE

Wendy K. Sherman 7/19/01
SPONSOR'S REPRESENTATIVE DATE

QA KH 7-17-01

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 5

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: November 06, 2000

AMENDMENT: Test Termination, Page-6-

DELETE: The contents of the reference and treatment group test chambers will be air dried at room temperature and then homogenized using a motar and pestle.

INSERT: The contents of the reference and treatment group test chambers will be air dried at room temperature and then homogenized.

REASON: Typographical error in protocol.

AMENDMENT: Test Termination, Page-6-

DELETE: The contents of the reference and treatment group test chambers will be air dried at room temperature and then homogenized. Aliqouts of the dried sediments will be analyzed for DBDPO and metabolites (if any). The chemical analysis of the samples will be performed by Wildlife International, Ltd. The analytical methods used will be based upon methodology developed in consultation with the Sponsor and will be amended to the protocol.

INSERT: The contents of the entire reference and treatment group test chambers (including the additional treatment group not affixed to a mineralization apparatus) will be air dried at room temperature and then homogenized. Aliquots of the homogenized Day 0 and Week 32 dried sediments will be analyzed for trace level lower brominated diphenyl oxides by AXYS Analytical Services Ltd. (Sidney, British Columbia, Canada). Additionally, seven replicate samples from each of the Day 0 and Week 32 homogenates will be analyzed for DBDPO by Wildlife International, Ltd using HPLC. The analytical methods used by Wildlife International, Ltd. will be based upon methodology developed in consultation with the Sponsor and will be amended to the protocol. The extracted samples analyzed for DBDPO at Wildlife International, Ltd. will be combusted using a Packard oxidizer (or equivalent) to determine residual radioactivity (if any) and extraction efficiency. If degradation is deemed to have occurred, analysis of additional samples will be initiated by protocol amendment.

REASON: Additional analyses requested by the Sponsor's Representative.

P.C. Lepp
STUDY DIRECTOR

7-17-01
DATE

LABORATORY MANAGEMENT

7/17/01
DATE

Wendy K. Sherman
SPONSOR'S REPRESENTATIVE

7/19/01
DATE

QA KH 7-17-01

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 6

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: November 06, 2000

AMENDMENT: Mass Balance Determination, Page-7-

DELETE: Three replicate samples of the dried sediments will be combusted using a Packard oxidizer (or equivalent).

INSERT: Seven replicate samples of the dried sediments will be combusted using a Packard oxidizer (or equivalent). Samples from the oxidizer will be analyzed by liquid scintillation counting to determine the amount of radioactivity associated with the dried sediments.

REASON: Seven replicates of the dried sediments were analyzed to generate a statistically significant mean for the radioactivity present in the samples.

STUDY DIRECTOR — DATE 7/20/2001

LABORATORY MANAGEMENT — DATE 7/20/01

SPONSOR'S REPRESENTATIVE — DATE 7/23/01

QA KH 7-20-01

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 7

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: April 02, 2001

AMENDMENT: Calculations, Page-7-

INSERT: The measured DBDPO concentrations will be converted to a DBDPO mass based on the actual dry weight of the sediment and the measured mass will be compared to the mass of DBDPO added at test initiation.

REASON: The conversion was performed to assess whether the measured concentrations were different from the starting concentrations.

P.C. Lamp — STUDY DIRECTOR — 7-17-01 — DATE

K. K. — LABORATORY MANAGEMENT — 7/17/01 — DATE

QA KH 7-17-01

Wendy K. Sherman — SPONSOR'S REPRESENTATIVE — 7/19/01 — DATE

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 8

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: April 02, 2001

AMENDMENT: Treatment of Results, Page-7-

DELETE: Statistics beyond the calculation of standard deviations and means will not be used in the evaluation of the results.

INSERT: The average measured DBDPO concentrations of the day-0 and week-32 test sediments will be statistically analyzed. In addition, the differences between the DBDPO mass weighed into the test chambers on day-0 and the DBDPO mass calculated using the measured DBDPO concentration at week-32 will statistically analyzed.

REASON: Statistical analysis will be used to determine if the concentrations of DBDPO in the test sediments at the start and conclusion of the study were significantly different.

P.C. Lamb — STUDY DIRECTOR — 7-17-01 — DATE

LABORATORY MANAGEMENT — 7/17/01 — DATE

QA KH 7-17-01

Wendy K. Sherman — SPONSOR'S REPRESENTATIVE — 7/19/01 — DATE

PROJECT NO.: 439E-104
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WILDLIFE INTERNATIONAL LTD.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **AMENDMENT NO.:** 9

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

PROJECT NO.: 439E-104

EFFECTIVE DATE: March 07, 2001

AMENDMENT: For Laboratory Use Only, Page-2-

Change the reference substance number from 4771 to 5189 & 5194.

REASON: The reference substance initially indicated in the protocol was consumed prior to the start of the study. In addition, both radiolabelled and non labeled forms of the reference substance were used in the study.

P.C. Lamp — STUDY DIRECTOR — 7-17-01 — DATE

LABORATORY MANAGEMENT — 7/17/01 — DATE

Wendy K. Sherman — SPONSOR'S REPRESENTATIVE — 7/19/01 — DATE

QA KH 7.17.01

WILDLIFE INTERNATIONAL LTD.

PROJECT NO.: 439E-104
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: POTENTIAL FOR BIOTRANSFORMATION OF RADIOLABELLED DECABROMODIPHENYL OXIDE (DBDPO) IN ANAEROBIC SEDIMENT

PROTOCOL NO.: 439/111099/MAS/SUB439 **DEVIATION NO.:** 1

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439E-104

DEVIATION: Test Inoculum, Page -5-

The 2 mg resazurin/L solution (surface water) was prepared at 0.2 mg resazurin/L solution (surface water).

REASON: Inadvertent error

IMPACT: In the best judgment of the Study Director, this deviation did not impact the integrity of study. The indicator's color in solution was readily visible under aerobic and anaerobic conditions.

STUDY DIRECTOR — DATE 3/22/2000

LABORATORY MANAGEMENT — DATE 3/23/00

DECABROMODIPHENYL OXIDE (DBDPO): A TOXICITY TEST TO DETERMINE THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE OF SIX SPECIES OF PLANTS

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439-101

OECD Guideline for Testing of Chemicals
Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests

and

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.4100 and 850.4225

AUTHORS:

John R. Porch
Henry O. Krueger, Ph.D.

STUDY INITIATION DATE: January 26, 2001

STUDY COMPLETION DATE: August 3, 2001

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Decabromodiphenyl Oxide (DBDPO): A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439-101

STUDY COMPLETION: August 3, 2001

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

STUDY DIRECTOR:

John R. Porch — 3 Aug 2001

John R. Porch — Date

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all audits and inspections and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY	DATE CONDUCTED	STUDY DIRECTOR	MANAGEMENT
Test Substance Preparation and Application	March 27, 2001	March 27, 2001	March 27, 2001
Dry Weights	April 20, 2001	April 20, 2001	April 20, 2001
Data Entry	May 2-3, 2001	May 3, 2001	May 10, 2001
Biological Data and Draft Report	July 16-17, 2001	July 17, 2001	July 25, 2001
Final Report	August 3, 2001	August 3, 2001	August 3, 2001

Marshall T. Hynson
Quality Assurance Program Supervisor

8/3/2001
Date

REPORT APPROVAL

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: DECABROMODIPHENYL OXIDE (DBDPO): A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439-101

STUDY DIRECTOR:

John R. Porch — 3 Aug 2001
John R. Porch — Date
Supervisor, Non-Target Plants and Insects

CHEMISTRY PRINCIPAL INVESTIGATOR:

Timothy Z. Kendall — 8/3/01
Timothy Z. Kendall — Date
Supervisor

WILDLIFE INTERNATIONAL, LTD. MANAGEMENT:

[signature] — 8/3/01
Henry O. Krueger, Ph.D. — Date
Director, Aquatic Toxicology and Non-Target Plants

Willard B. Nixon — 8/3/01
Willard B. Nixon, Ph.D. — Date
Director, Analytical Chemistry

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TABLES

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(continued)

APPENDICES

SUMMARY

WILDLIFE INTERNATIONAL, LTD. PROJECT NO: 439-101

TEST SUBSTANCE: Decabromodiphenyl Oxide

STUDY TITLE: Decabromodiphenyl Oxide (DBDPO): A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

GUIDELINES: OECD Guideline for Testing of Chemicals, Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests
OPPTS 850.4100 (Public Draft)
OPPTS 850.4225 (Public Draft)

NOMINAL TEST LEVELS: 0 (Control), 391, 781, 1563, 3125, and 6250 mg/kg dry soil

TEST DATES:

STUDY INITIATION:	January 26, 2001
Experimental Start (OECD):	March 27, 2001
Experimental Start (EPA):	March 28, 2001
Experimental Termination:	April 20, 2001
STUDY COMPLETION:	August 3, 2001

LENGTH OF TEST: 21 days

TEST SPECIES: Corn (*Zea mays*), Cucumber (*Cucumis sativa*), Onion (*Allium cepa*), Ryegrass (*Lolium perenne*), Soybean (*Glycine max*), Tomato (*Lycopersicon esulentum*)

RESULTS: The soil incorporation of Decabromodiphenyl Oxide caused no effects on emergence, survival, or growth on any of the six plant species tested. Therefore, the highest soil concentration tested, 6250 mg/kg, was considered to be the NOEC for these test species.

INTRODUCTION

This seedling emergence study was conducted for American Chemistry Council's Brominated Flame Retardant Industry Panel at the Wildlife International, Ltd. greenhouse facility in Easton, Maryland. The in-life portion of the test was conducted from March 28, 2001 to April 18, 2001. Raw data generated at Wildlife International, Ltd., the study protocol, and a copy of the final report were filed in the archives located on the Wildlife International, Ltd. site. Key personnel involved in the study are listed in Appendix 1.

PURPOSE

The purpose of the study was to determine the effects of decabromodiphenyl oxide on the seedling emergence and growth of six species of non-target plants.

EXPERIMENTAL DESIGN

The experimental design for this study consisted of a negative control and five treatment groups. Each group had four replicate pots with ten seeds planted in each pot. Test concentrations of decabromodiphenyl oxide were made by soil incorporation to each treatment group prior to the planting of seeds. The nominal test substance concentrations were 391, 781, 1563, 3125, and 6250 mg of DBDPO per kilogram of dry soil (mg/kg). A control group, which received no test substance incorporation, was maintained concurrently.

Seeds were impartially assigned to prelabelled growth pots on the day of test initiation. The replicate pots were placed in a randomized block design on a greenhouse table after planting. Observations of emergence and general assessments of seedling condition were made on Days 7, 14, and 21, while observations of height, shoot dry weight, and assignment of plant condition scores were made only on Day 21.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Decabromodiphenyl Oxide: A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants" (Appendix 2). The methods used in conducting this study were based upon procedures specified in the OECD Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests (1) and the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Numbers 850.4100 (2) and 850.4225 (3).

Test Substance

The test substance consisted of a composite of decabromodiphenyl oxide samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International, Ltd. Identification Number
Albemarle Corporation	4730-IL	October 15, 1998	4663
Great Lakes Chemical Corporation	848ODI30B	October 19, 1998	4664
Bromine Compounds Ltd.	980077	October 21, 1998	4667

The composite test substance was assigned Wildlife International, Ltd. identification number 4700 and was stored under ambient conditions. The composite test substance was shipped to Albemarle Corporation for characterization and purity analyses (Appendix 3). The results of the analyses indicated the composite test substance was homogeneous. The conclusion of the characterization was that the test article was decabromodiphenyl oxide with a purity of 97.9%.

Preparation and Soil Incorporation of Test Substance

The test soil was prepared by mixing decabromodiphenyl oxide into bulk test soil with a measured soil moisture of 20%. A soil pre-mix was prepared by adding five known weights (19.9, 39.8, 79.7, 159.4, and 318.8 g) of decabromodiphenyl oxide to soil for a total weight of 1 kg. The pre-mix was then mixed overnight on an end over end mixer. The next morning, approximately 59 kg of bulk soil was measured into a soil mixer, and approximately 1 kg pre-mix was added for each test concentration. The test substance and bulk soil were then mixed for twenty minutes in order to prepare the test soil for each treatment group. Soils were mixed from lowest to highest concentration to avoid cross-contamination. The negative control pre-mix and test soil were prepared in the same manner as the other test groups, but no test substance was added. At the completion of mixing, the test soils were sampled to provide material for analytical confirmation of the test concentrations. Analytical samples were stored frozen after their collection until they were analyzed.

Test Species

The common and scientific names for the six species tested, the seed source, and their approximate planting depths are listed below:

Test Species / Variety:	Seed Source:	Planting Depth
Corn (*Zea mays*) / Mandan Bride	Johnny's Selected Seeds, Albion, ME, USA	20 mm
Onion (*Allium cepa*) / Texas Grano	Territorial Seed Co., Cottage Grove, OR, USA	6 mm
Ryegrass (*Lolium perenne*) / Manhattan III	Meyer Seed Co., Baltimore, MD, USA	6 mm
Cucumber (*Cucumis sativa*) / Straight Eight	Meyer Seed Co., Baltimore, MD, USA	20 mm
Soybean (*Glycine max*) / Green Envy	Johnny's Selected Seeds, Albion, ME, USA	20 mm
Tomato (*Lycopersicon esculentum*) /Rutgers	Meyer Seed Co., Baltimore, MD, USA	6 mm

These species were chosen because they represent ecologically important families, and are readily cultivated test organisms that are widely used in research. Seeds were selected from a single size class within each species in order to reduce the potential for bias from differing seed sizes. Seeds used in this study were not treated with fungicides, insecticides or repellents prior to test initiation.

Test Soil

The soil used for the test represented a loamy sand soil, and was composed of kaolinite clay, industrial quartz sand, and peat mixed in a 4:50:5 ratio (w:w:w). Crushed limestone was added to buffer the pH of the soil, and a slow-release fertilizer was added to provide nutrients essential for plant growth. A sample of soil representative of that used in this study was sent to Agvise Laboratories, Inc., in Northwood, North Dakota, for analysis of the particle size distribution and organic matter content of the soil. The soil was determined to consist of 84% sand, 8% silt, and 8% clay, with an organic matter content of 2.8%. The soil pH was measured to be 7.7. A copy of the complete report from Agvise Laboratories, Inc. was filed in the archives at Wildlife International, Ltd. along with the raw data for this study.

Planting of Seeds

Seeds were planted in plastic pots (approximately 16 cm in diameter and 11 cm deep) on the day of test substance application. A template was used to gently compact the soil and leave ten uniform holes for planting. One indiscriminately selected seed was then planted in each hole, for a total of ten seeds in each pot. Holes were then closed by slightly depressing the soil surface.

Watering of Seedlings

Initial watering was done to the soil surface after planting. Water lost through transpiration and evaporation was replaced by subirrigation with well water from the greenhouse facility. Seedlings were subirrigated to minimize the potential for the leaching of the test substance through the soil. Subirrigation trays were filled to a predetermined depth to help standardize the amount of water delivered to each tray. The days on which watering occurred are listed in Appendix 3.

Environmental Conditions

The environmental conditions (temperature and relative humidity) of the test are summarized in Appendix 3. The temperature within the greenhouse was controlled with a Wadsworth MicroStep S/A Environmental Control System. Artificial lighting (high pressure sodium) was used to supplement natural sunlight in order to provide a uniform 14-hour photoperiod. The temperature and relative humidity within the greenhouse were continuously monitored during the test with the Wadsworth control system.

Pesticide and Metal Screening of Well Water and Soil

The well water and soil used for plant studies are analyzed periodically for pesticide and metals. No analytes were measured at levels that were expected to have an impact on the study. Reports for the latest analyses are stored in the archives at the Wildlife International, Ltd. site in Easton, Maryland.

Observations and Measurements

Observations on Days 7 and 14 were made to document seedling emergence. Observations on Day 21 were made to document seedling emergence and growth, and to determine changes in the general condition of seedlings following the application of the test substance. Observations consisted of noting whether emergence had or had not occurred, and assessing the condition of each seedling. Emergence was defined as the presence of visible plant tissue at the surface of the soil. Seedling condition was described by noting the presence or absence of possible signs of phytotoxicity such as necrosis, leaf wrinkle, chlorosis, plant lodging or plant stunting. Each emerged seedling was then assigned a numerical score (see Table 1) that described the plant condition (4). Condition score is a subjective or qualitative assessment that determines whether damage is slight, moderate, or severe. A score of 10 does not mean that 10% of the plant is

showing the effect (e.g. chlorosis), merely that the severity of the effect (e.g. chlorosis) is very slight.

The growth of emerged seedlings was evaluated by assessing the height and dry weight of living seedlings at test termination. Seedling height was measured to the nearest whole centimeter from the surface of the soil to the tip of the tallest leaf (corn, onion, and ryegrass) or to the apical meristem (cucumber, soybean, and tomato). Seedlings were then clipped at soil level; the shoots of all living seedlings within a replicate were placed in a labeled bag, and dried. The total dry weight of the replicate was determined, and the mean weight per plant was calculated by dividing the total weight by the number of seedlings weighed.

Analytical Chemistry

On the day of test soil preparation, three soil samples were collected from the 391, 781, 1563, 3125, and 6250 mg/kg treatment groups to verify the test concentrations and determine the homogeneity of the test substance in the carrier (soil). One sample was collected from the control group. Samples were placed in a freezer upon collection on March 28, 2001, and stored frozen until analysis on May 24, 2001. Chemical analysis of the soil used in this study was performed by Wildlife International, Ltd. (Appendix 4). The test substance was used to prepare calibration standards.

Data Analyses

Statistical analyses were used to aid in the evaluation of effects of test substance application on seedling emergence, survival, mean shoot weight, and seedling height. These variables were defined for statistical analysis as follows:

Seedling Emergence:

The number of emerged seedlings per ten planted seeds in each pot.

Survival:

The number of emerged seedlings in each pot that were living at test termination per ten planted seeds.

Mean Shoot Weight:

The average dry shoot weight of living emerged seedlings in each pot.

Height:

The average height of living emerged seedlings in each pot.

Mean seedling emergence, survival, weight, and height of the control and treatment groups were compared with Dunnett's t-test, using the DUNNETT option of the GLM (general linear model) procedure of SAS version 8 (5). Significance was determined at the level of 0.05 ($p<0.05$).

Additionally, test data were evaluated to determine the no-observed-effect-concentration (NOEC) and lowest-observable-effect-concentration (LOEC) for condition and growth. The NOEC is defined as the maximum test substance concentration that shows no adverse phytotoxic effects and below which no phytotoxic effects are manifested. The LOEC is defined as the lowest test substance concentration used in the study that shows an adverse effect on a variable of interest. Dunnett's test was used to aid in establishing the NOEC by determining which treatment groups differed significantly from the control group.

RESULTS AND DISCUSSION

Analytical Chemistry

The results of analyses to measure concentrations of decabromodiphenyl oxide in the soil samples collected during the test are presented in Appendix 4.

Biological Results

The results of the test are summarized for each species in Tables 2 through 7. Complete results are presented by species in Appendices 6 through 11. There were no apparent effects on any endpoint for any of the six species tested. Statistical analyses indicated that there were no significant differences (Dunnett's test, $p>0.05$) between the control and treatment group mean emergence, survival, height, or weight for corn, cucumber, ryegrass, and onion. On day 21, soybean showed significant differences (Dunnett's test, $p<0.05$) between control and the 1563 mg/kg treatment group mean emergence, survival, and height. On day 21, tomato showed significant differences (Dunnett's test, $p<0.05$) between control and the 391 mg/kg treatment group mean emergence, and survival. These significant differences were not considered dose-responsive, and not attributable to treatment. Additionally, there were no signs of treatment-related phytotoxicity observed on seedlings of any species at any test concentration.

CONCLUSIONS

No effects on seedling emergence, survival, or growth were observed on any of the six plant species tested. Therefore, the NOEC for emergence and growth of all seedlings in this study was determined to be 6250 mg/kg, which was the highest soil concentration tested.

REFERENCES

1 **OECD Guideline for Testing of Chemicals.** 1998. *Guideline for Testing of Chemicals, Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests*. Organization for Economic Cooperation Development

2 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence).

3 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4225: Terrestrial Plant Toxicity, Tier II (Seedling Emergence).

4 **Frans, Robert E. and Ronald E. Talbert.** 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 *in* B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama.

5 **SAS Institute, Inc.** 1989. SAS/STAT User's Guide , Version 6, Fourth Edition, Volume 1, Cary, NC, SAS Institute, Inc., 943 pp.

Table 1

Seedling Condition Rating System

The rating system below was used to help evaluate the health of seedlings on Day 21. Assigned scores by treatment group are reported on the following pages.

Rating	Category	Description
0	No Effect	No noticeable effect
10		Effect barely noticeable
20	Slight Effect	Some effect, not apparently detrimental
30		Effect more pronounced, not obviously detrimental
40		Effect moderate, plants appear able to recover
50	Moderate Effect	More lasting effect, recovery somewhat doubtful
60		Lasting effect, recovery doubtful
70		Heavy injury, loss of individual leaves
80	Severe Effect	Plant nearly destroyed, a few surviving leaves
90		Occasional surviving leaves
100	Complete Effect	Death of entire plant

Rating scale adapted from:

Frans, Robert E. and Ronald E. Talbert. 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 in B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama.

Table 2

Effects of Decabromodiphenyl Oxide on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with CORN

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (g) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	9.75 ± 0.50	9.75 ± 0.50	9.75 ± 0.50	9.75 ± 0.50	0.5248 ± 0.0389	44.8 ± 2.56
391	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	0.5433 ± 0.1232 (-4%)	46.4 ± 4.71 (-4%)
781	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	9.50 ± 0.58 (3%)	0.5507 ± 0.0761 (-5%)	44.4 ± 6.60 (1%)
1563	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	9.50 ± 0.58 (3%)	0.6994 ± 0.0543 (-33%)	53.4 ± 2.86 (-19%)
3125	9.50 ± 0.58 (3%)	9.50 ± 0.58 (3%)	9.50 ± 0.58 (3%)	9.50 ± 0.58 (3%)	0.6637 ± 0.0359 (-26%)	50.1 ± 2.21 (-12%)
6250	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	9.75 ± 0.50 (0%)	9.50 ± 0.58 (3%)	0.5707 ± 0.1524 (-9%)	46.5 ± 7.15 (-4%)

Table 3

Effects of Decabromodiphenyl Oxide on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with CUCUMBER

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (g) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	9.25 ± 0.96	9.25 ± 0.96	9.25 ± 0.96	9.25 ± 0.96	0.4423 ± 0.0348	14.4 ± 2.66
391	9.75 ± 0.50 (-5%)	9.75 ± 0.50 (-5%)	9.75 ± 0.50 (-5%)	9.75 ± 0.50 (-5%)	0.3954 ± 0.0600 (11%)	13.4 ± 3.24 (7%)
781	9.75 ± 0.50 (-5%)	9.75 ± 0.50 (-5%)	10.00 ± 0.00 (-8%)	10.00 ± 0.00 (-8%)	0.4448 ± 0.0497 (-1%)	15.3 ± 1.56 (-7%)
1563	10.0 ± 0.00 (-8%)	10.00 ± 0.00 (-8%)	10.00 ± 0.00 (-8%)	10.00 ± 0.00 (-8%)	0.3756 ± 0.0523 (15%)	13.5 ± 0.87 (6%)
3125	9.75 ± 0.50 (-5%)	10.00 ± 0.00 (-8%)	10.00 ± 0.00 (-8%)	10.00 ± 0.00 (-8%)	0.4561 ± 0.0501 (-3%)	15.9 ± 1.92 (-10%)
6250	7.50 ± 5.00 (19%)	7.50 ± 5.00 (19%)	7.50 ± 5.00 (19%)	7.50 ± 5.00 (19%)	0.4144 ± 0.0183 (6%)	15.0 ± 1.70 (-5%)

Table 4

Effects of Decabromodiphenyl Oxide on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with ONION

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (mg) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	6.75 ± 2.22	7.75 ± 2.22	7.75 ± 2.22	7.75 ± 2.22	8.02 ± 2.34	6.9 ± 1.33
391	6.50 ± 3.0 (4%)	9.00 ± 1.41 (-16%)	9.00 ± 1.41 (-16%)	9.00 ± 1.41 (-16%)	6.40 ± 0.84 (20%)	6.0 ± 0.73 (14%)
781	5.50 ± 2.08 (19%)	8.50 ± 1.00 (-10%)	8.25 ± 1.26 (-6%)	7.75 ± 0.96 (0%)	6.41 ± 0.95 (20%)	6.0 ± 0.66 (13%)
1563	8.25 ± 1.71 (-22%)	9.00 ± 1.41 (-16%)	9.00 ± 1.41 (-16%)	8.50 ± 1.29 (-10%)	7.08 ± 0.66 (12%)	7.1 ± 0.98 (-3%)
3125	8.50 ± 1.29 (-26%)	9.00 ± 1.41 (-16%)	9.00 ± 1.41 (-16%)	8.75 ± 1.26 (-13%)	9.58 ± 1.64 (-20%)	7.7 ± 1.20 (-12%)
6250	8.75 ± 0.50 (-30%)	8.75 ± 0.50 (-13%)	8.75 ± 0.50 (-13%)	8.50 ± 0.58 (-10%)	8.28 ± 0.92 (-3%)	8.1 ± 0.86 (-18%)

Table 5

Effects of Decabromodiphenyl Oxide on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with RYEGRASS

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (mg) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	9.00 ± 1.15	9.00 ± 1.15	9.00 ± 1.15	8.75 ± 0.96	22.2 ± 3.83	13.1 ± 0.59
391	9.25 ± 0.50 (-3%)	9.25 ± 0.50 (-3%)	9.25 ± 0.50 (-3%)	9.25 ± 0.50 (-6%)	18.1 ± 2.57 (19%)	12.0 ± 0.74 (8%)
781	9.25 ± 0.96 (-3%)	9.25 ± 0.96 (-3%)	9.25 ± 0.96 (-3%)	9.25 ± 0.96 (-6%)	23.3 ± 4.85 (-5%)	13.1 ± 1.06 (0%)
1563	8.50 ± 1.29 (6%)	8.75 ± 0.96 (-3%)	8.75 ± 0.96 (3%)	8.25 ± 0.96 (6%)	23.0 ± 3.03 (-3%)	12.1 ± 0.93 (8%)
3125	9.50 ± 0.58 (-6%)	9.75 ± 0.50 (-8%)	9.75 ± 0.50 (-8%)	9.50 ± 0.58 (-9%)	29.4 ± 3.89 (-32%)	15.4 ± 1.52 (-17%)
6250	9.75 ± 0.50 (-8%)	9.75 ± 0.50 (-8%)	10.00 ± 0.00 (-11%)	10.00 ± 0.00 (-14%)	23.8 ± 5.28 (-7%)	13.9 ± 1.86 (-6%)

Table 6

Effects of Decabromodiphenyl Oxide on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with SOYBEAN

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (g) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	0.7058 ± 0.971	31.2 ± 5.07
391	10.00 ± 0.00 (0%)	10.00 ± 0.00 (0%)	10.00 ± 0.00 (0%)	9.75 ± 0.50 (3%)	0.6555 ± 0.055 (7%)	32.1 ± 5.95 (-3%)
781	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	0.6734 ± 0.0475 (5%)	32.7 ± 0.71 (-5%)
1563	7.25 ± 2.06* (28%)	8.00 ± 1.41* (20%)	8.00 ± 1.41* (20%)	8.00 ± 1.41* (20%)	0.6337 ± 0.1063 (10%)	20.7 ± 3.57* (34%)
3125	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	0.6854 ± 0.0513 (3%)	32.3 ± 3.57 (-3%)
6250	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	0.6845 ± 0.0638 (3%)	30.7 ± 1.84 (2%)

* Treatment group mean is significantly different from the control mean (Dunnett's test $p<0.05$).

Table 7

Effects of Decabromodiphenyl Oxide on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with TOMATO

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (g) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	5.00 ± 1.41	9.00 ± 0.82	9.25 ± 0.50	9.25 ± 0.50	0.0466 ± 0.0172	6.2 ± 0.83
391	3.50 ± 1.00 (30%)	6.75 ± 0.50* (25%)	7.00 ± 0.00* (24%)	7.00 ± 0.00* (24%)	0.0359 ± 0.0057 (23%)	5.6 ± 0.74 (9%)
781	6.00 ± 1.41 (-20%)	8.75 ± 0.50 (3%)	9.25 ± 0.50 (0%)	9.25 ± 0.50 (0%)	0.04064 ± 0.0150 (13%)	5.9 ± 0.88 (5%)
1563	6.00 ± 1.63 (-20%)	8.25 ± 1.50 (8%)	8.25 ± 1.50 (11%)	8.25 ± 1.50 (11%)	0.0346 ± 0.0038 (26%)	5.7 ± 0.21 (9%)
3125	5.75 ± 1.26 (-15%)	9.00 ± 0.82 (0%)	9.50 ± 0.58 (-3%)	9.25 ± 0.50 (0%)	0.0759 ± 0.0164 (-63%)	7.4 ± 0.49 (-19%)
6250	5.75 ± 1.71 (-15%)	8.75 ± 0.50 (3%)	8.75 ± 0.50 (5%)	8.75 ± 0.50 (5%)	0.0735 ± 0.0142 (-58%)	6.8 ± 0.43 (-11%)

* Treatment group mean is significantly different from the control mean (Dunnett's test, $p<0.05$).

Appendix 1

Personnel Involved In the Study

The following key personnel were involved in the conduct or management of this study:

(1) Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
(2) John R. Porch, Supervisor, Non-Target Plants and Insects
(3) Andrew J. Brignole, Biologist
(4) Timothy Z. Kendall, Supervisor
(5) Willard B. Nixon, Director, Analytical Chemistry

Appendix 2

Study Protocol, Amendments, and Deviations

PROTOCOL

DECABROMODIPHENYL OXIDE (DBDPO): A TOXICITY TEST TO DETERMINE THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE OF SIX SPECIES OF PLANTS

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.4100 and 850.4225

and

OECD Guideline for Testing of Chemicals
Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

January 10, 2001

Wildlife International, Ltd.

- 2 -

DECABROMODIPHENYL OXIDE (DBDPO): A TOXICITY TEST TO DETERMINE THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE OF SIX SPECIES OF PLANTS

SPONSOR:	American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	John R. Porch Senior Biologist
LABORATORY MANAGEMENT:	Henry O. Krueger, Ph.D. Director of Aquatic Toxicology & Non-Target Plants

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: February 14, 2001 Experimental Termination Date: March 14, 2001

Project No.: 439-101

Test Concentrations: 391, 781, 1563, 3125 and 6250 mg/kg dry soil

Test Substance No.: 4700 Reference Substance No. (if applicable): NA

PROTOCOL APPROVAL

John R. Porch — STUDY DIRECTOR — 26 Jan 2001 — DATE

H. K. — LABORATORY MANAGEMENT — 2/2/01 — DATE

Wendy K. Sherman — SPONSOR'S REPRESENTATIVE — 1/15/01 — DATE

PROTOCOL NO. 439/011001/SEEDEM-10/SU439

Wildlife International, Ltd.

INTRODUCTION

Wildlife International, Ltd. will conduct a toxicity test with six species of plants to determine the effects of a test substance on seedling emergence and early growth. The test will be conducted at the Wildlife International, Ltd. plant testing facility near Easton, Maryland. The six species to be tested include rye grass, onion, corn, soybean, cucumber, and tomato. The study will be performed based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.4100 (1) and 850.4225 (2) and in the OECD Guideline for Testing of Chemicals: Proposal for Revision of Guideline 208: "Terrestrial Non-target Plant Tests" (3). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to determine the effect of a test substance on the seedling emergence and growth of six species of plants.

EXPERIMENTAL DESIGN

The target test concentration(s) will be selected by the Sponsor in consultation with Wildlife International, Ltd., and will be based upon information such as the results of exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance or other relevant information. If necessary, the test concentrations to be used for each species will be added to the protocol by amendment.

For each plant species tested, seeds will be planted and exposed to a series of five concentrations of the test substance. A negative control and, if appropriate, a solvent control group will be maintained concurrently. There will be four replicates for each treatment and control group. Each replicate will consist of a growth pot containing ten seeds. The replicates will be placed on a benchtop in a greenhouse according to a randomized design. Data collected from all replicates within a treatment group will be combined for calculating EC25 and EC50 values, as well as the no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC).

Wildlife International, Ltd.

One application of each of the various treatments will be made by soil incorporation of the test substance prior to planting seeds. The duration of the in-life portion of the test will be 21 days following planting, during which time possible phytotoxic effects of the test substance on seedling emergence and growth of emerged seedlings will be evaluated.

MATERIALS AND METHODS

Test Substance

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLPs), 40 CFR Part 160.31. The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test substance has been characterized according to GLPs prior its use in the study. If written verification of GLP test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report. The attached form **IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR** (Appendix I) is provided to assist the sponsor in providing this information.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Test Soil Preparation

Concentrations of the test substance in the soil will be prepared on a dry weight basis (e.g., mg test chemical/kg dry soil). The test substance will be incorporated into the soil for each treatment level prior to planting.

Species to be Tested

The six species of plants used in this study were chosen because they are economically important, and are readily cultivated test organisms that are widely used in research. The common and scientific names for the species and their approximate planting depths are listed below:

Wildlife International, Ltd.

		Planting Depth
Monocots:		
Rye Grass	*Lolium perenne*	6 mm
Onion	*Allium cepa*	6 mm
Corn	*Zea mays*	2.0 - 2.5 cm
Dicots:		
Soybean	*Glycine max*	2.0 - 2.5 cm
Cucumber	*Cucumis sativa*	2.0 - 2.5 cm
Tomato	*Lycopersicon esculentum.*	6 mm

Seeds will be selected from a single size class within each species. The seeds of most plant species are sorted according to size by the supplier prior to being obtained by Wildlife International, Ltd. However, in some cases it may be necessary to further sort seeds to form a more uniform size class that reduces the potential for bias from differing seed sizes.

Seeds used in this study will not have been treated with fungicides, insecticides or repellents prior to test initiation. Seeds will be obtained from a producer or supplier such as Meyer Seed Company, Baltimore, Maryland. Any documentation provided from the supplier concerning the identification and history of the seeds used will be included in the study data.

Test Soil

Test plants will be grown in pots with a sandy-loam soil substrate. Analyses will be performed at least once annually to characterize the soil. A sample of soil representative of that used in this study will be sent to Agvise Laboratories, Inc., in Northwood, North Dakota, for analysis of the particle size distribution and organic matter content of the soil. Soil characterization will include, but may not be limited to, the determination of particle size distribution, organic matter content, and pH. Those items relevant to the conduct of the study will be discussed in the final report. The complete report from Agvise Laboratories, Inc. will be filed in the archives located at Wildlife International, Ltd. The results of the characterization will be stored in the archives located at the Wildlife International, Ltd. site, and those items relevant to the conduct of the study will be discussed in the final report.

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Pesticide and Metal Screening

Neither the well water nor the artificial soil are expected to have contaminants present in quantities known to be capable of interfering with the study. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of water and soil used in this study. Results of the analyses will be stored in the archives located on the Wildlife International Ltd. site.

Environmental Conditions

The test will be conducted within a greenhouse. Environmental conditions, including temperature and light intensity, will be controlled using a Wadsworth MicroStep/SA environmental control system. Temperature and relative humidity in the study room will be continuously monitored with a Campbell Scientific data logger, and daily conditions throughout the test will be reported. A photoperiod of at least 14 hours light will be maintained during the test. Artificial lighting may be used to lengthen short-day photoperiods or to supplement natural sunlight on overcast days.

Test Procedure

Growth pots will be filled with test or control soil, and ten seeds of one species will be planted per replicate. The seeds will be planted at the appropriate depth and will be approximately equally spaced. Seeds will be assigned to test and control groups and planted in growth pots uniquely identified with a minimum of the species name, project number, treatment group designation, and replicate. This method of application was chosen because contaminated soil is the most likely route of exposure to plants. After planting, the growth pots will be placed on benches in the greenhouse in a randomized configuration to minimize bias from microclimates which may exist within the greenhouse. Initial watering will be done to the soil surface after planting. Thereafter, water will be supplied to the growth pots by sub-irrigation to help ensure that sufficient water is available for seedling growth. Records of the days that watering occurs and source of water used will be kept in the study data.

The growth pots will be observed weekly after test initiation in order to determine the number of emerged seedlings. The in-life portion of the test will terminate twenty-one days after initiation, however, the test may be extended at the discretion of the study director for one or more species. If

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any portion of the test is extended, the duration of and the reason for the extension will be documented in the data and discussed in the final report. At the termination of the in-life portion of the test, height measurements and the condition of the emerged seedlings will be recorded. The height of each living seedling within a replicate will be determined in order to calculate the mean seedling height per replicate. The exact method used to measure height may vary with species, and will be described in the raw data and included in the final report.

At the in-life phase termination, the condition of seedlings will be assessed utilizing a rating system based upon Frans and Talbert (4). A numerical rating will be assigned to help characterize changes in the seedlings' morphology including necrosis, chlorosis, general development, or any other characteristic that may be deemed a response of the seedling to the treatment. Ratings may range from 0 to 100, 0 indicating normal seedling appearance, 100 indicating emerged seedlings that have died prior to test termination. Intermediate scores reflect the severity of changes in plant condition. After final observations are completed, plants will be clipped at soil level and the above-ground portion (shoots) of all living plants within each replicate will be dried to a constant weight. The mean shoot dry weight of each replicate will be calculated.

Sampling for Analytical Measurements

On each day of test substance application, samples of the test soils will be collected for the analysis of the test substance. Samples will be placed in an appropriate storage container (e.g., glass or polypropylene bottles) and stored under conditions designated by the Sponsor until analyzed. Triplicate samples will be collected from the soil of each test concentration to verify concentrations and demonstrate homogeneity in the soil.

Experimental Group	Day 0
Control	1
Solvent Control (if needed)	1
Level 1-Low Concentration	3
Level 2	3
Level 3	3
Level 4	3
Level 5-High Concentration	3
Total Number of Samples = 17	

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The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical validation phase of the study.

Analytical Method Development and Verification

Wildlife International, Ltd. will develop appropriate analytical methods and validate them for Sponsor approval prior to their use in support of this study. If the Sponsor provides an analytical method, Wildlife International, Ltd. will demonstrate its validity to the Sponsor before being used in support of this study. All analytical methods accepted for use in this study will be added by protocol amendment and described in detail as an Appendix to the final report."

Analytical Chemistry

Chemical analysis of the samples will be performed by Wildlife International, Ltd. using High Performance Liquid Chromatography (HPLC). The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report. Maximum sample holding times, prior to analysis, will not exceed one week from the date of the collection of samples.

Data Analyses

This section includes proposed statistical analyses. Additional tests or analyses may be performed when warranted at the discretion of the Study Director or by Sponsor request.

An evaluation of potential effects of the test substance on seedling emergence, the growth of emerged seedlings, as characterized by shoot weight and height, and seedling condition will be made. Statistical analyses will include the determination of effect concentrations (EC estimates), and the determination of which treatment groups differ significantly from the control group(s).

The 25 and 50% effect concentrations and their 95% confidence intervals will be determined when warranted using an appropriate technique, such as Probit analysis or linear interpolation. When possible, EC estimates will be made for mean seedling emergence, mean shoot weight and height of seedlings at test termination.

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The data will be evaluated to determine the lowest-observed-effect concentration (LOEC), defined as the lowest concentration of test substance used in the study that shows an adverse effect on a variable of interest. The no-observed-effect-concentration (NOEC) will be defined as the maximum concentration which shows no adverse phytotoxic effects and below which no phytotoxic effects are manifested. Dunnett's two-tailed test will be used to determine significant differences from the control(s) at the 0.05 level of significance. Significant differences from the control, or their absence, may help establish the LOEC and NOEC.

All statistical analyses will be performed on a personal computer using commercially available statistical software programs (5, 6). The specific statistical tests and the programs used to perform the tests will be described in the final report of the study.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International, Ltd. will include but not be limited to:

1. Copy of signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Test soil calculation and preparation.
5. Observations.
6. The methods used to analyze test substance concentrations and the results of analytical measurements.
7. Statistical calculations, if applicable.
8. Test conditions (temperature, humidity, etc.).
9. Calibration records for application equipment.
10. Copy of final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report will include, but not be limited to, the following, when applicable.

1. Name and address of the facility performing the study.

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2. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. The test substance identification including name, chemical abstract number or code number, strength, purity, composition, and other information provided by the Sponsor.
5. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor.
6. A description of the methods used to conduct the test.
7. A description of the test species, including the source and scientific name.
8. A description of the preparation of the test solutions.
9. The methods used to allocate seeds to test substrates and begin the test, the number of seeds and replicates per treatment, and the duration of the test.
10. A description of circumstances that may have affected the quality or integrity of the data.
11. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
12. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from the analyses.
13. Statistical methods used to evaluate the data.
14. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
15. The location where raw data and final report are to be stored.
16. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
17. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed by the Study Director.

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CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160); OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

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APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): _____

Reference Standard (if applicable): Analytical Standard: N/A

Internal Standard: N/A

Test Substance Sample Code or Batch Number: _____

Test Substance Purity (% Active Ingredient): _____ Expiration Date: _____

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? Yes _____ No _____

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd.

Ambient

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes _____ No _____

Other pertinent stability information: _____

IV. Toxicity Information:

	Acute Oral LD50	Dietary LC50 Data
Rat	_____	Rat _____
Mouse	_____	Mouse _____
Mallard	_____	Mallard _____
Quail	_____	Quail _____

Other Toxicity Information (including findings of chronic and subchronic tests):

_____

_____

V. Classification of the Compound:

Insecticide _____ Herbicide _____ Fungicide _____

Microbial Agent _____ Economic Poison _____

Other: _____

WILDLIFE INTERNATIONAL LTD Page 1 of 1

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Decabromodiphenyl Oxide (DBDPO): A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

PROTOCOL NO.: 439/011001/SEEDEM-10/SU439 **AMENDMENT NO.:** 1

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439-101

EFFECTIVE DATE: March 28, 2001

AMENDMENT:

Change the Proposed Experimental Start and Termination dates to March 28 and April 25, 2001, respectively.

REASON:

The test initiation was delayed pending completion of the test substance characterization.

STUDY DIRECTOR — DATE 29 Mar 01

LABORATORY MANAGEMENT — DATE 3/30/01

SPONSOR'S REPRESENTATIVE — DATE 4/6/01

Reviewed by QA
JHC 3-29-01

Wildlife International, Ltd.

DEVIATION FROM STUDY PROTOCOL

STUDY TITLE: Decabromodiphenyl Oxide (DBDPO): A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

PROTOCOL NO.: 439/011001/SEEDEM-10/SU439 **DEVIATION NO.:** 1

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439-101

DATE(S) OF DEVIATION: March 28 to May 24, 2001

DEVIATION:
Analytical samples were stored frozen for more than one week prior to analysis.

REASON:
Analyses were conducted based on instrument availability. Frozen storage was considered a conservative method of preserving sample integrity. There is no adverse impact on the study as a result of this deviation.

DEVIATION:
The analytical method developed by Wildlife International, Ltd. was not amended to the protocol.

REASON:
The analytical method was developed prior to its use in the study, and is described in the report. The omission of a protocol amendment was oversight. There is no adverse impact on the study as a result of this deviation.

STUDY DIRECTOR — DATE 2 Aug 01

LABORATORY MANAGEMENT — DATE 8/2/01

Appendix 3
Certificate of Analysis

ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT

INTERIM REPORT ON THE CHEMICAL CHARACTERIZATION OF DECABROMODIPHENYL OXIDE (DBDPO) IN SUPPORT OF A STUDY OF "DECABROMODIPHENYL OXIDE: A TOXICITY TEST TO DETERMINE THE EFFECT OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE OF SIX SPECIES OF PLANTS"

I.	Reference Protocol Number:	DBDPOSEEDLING-01-26-2001
II.	Sponsor:	American Chemistry Council Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209 Study Monitor: Wendy K. Sherman
III.	Analytical Testing Facilities:	Albemarle Corporation Albemarle Technical Center 8000 GSRI Avenue Baton Rouge, LA 70820 Study Chemist: Paul F. Ranken, Ph. D.
IV.	Dates of Performance:	Study initiation date: January 26, 2001 Interim report issued: March 13, 2001
V.	Test Article:	Decabromodiphenyl oxide (WIL Test Substance 4700). The test article is a composite of commercial product from Albemarle Corporation, Great Lakes Chemical Corporation and Ameribrom (the Dead Sea Bromine Group). The composite was prepared by Wildlife International Ltd., Easton, MD 21601.
VI.	Objective/Methodology:	This study was initiated to confirm the identity of the test article, to determine the purity of the test article and to confirm the stability of the test article during the study of

"Decabromodiphenyl Oxide: a Toxicity Test to Determine the Effect of the Test Substance on Seedling Emergence of Six Species of Plants." The identity of the test article sample was confirmed by Fourier Transform Infrared Spectroscopy using SOP No. ARS 284-R4. In this procedure, the test article sample infrared spectrum was compared to a standard reference spectrum of decabromodiphenyl oxide. The reference infrared spectrum was located in the Aldrich Condensed Phase High Resolution data library. The data library is an electronic collection of infrared spectra given in the Aldrich Library of FT-IR Spectra monographs. The purity (area % decabromodiphenyl oxide) of the test article sample was determined by gas chromatography using SOP No. ARS 325-R1. In this procedure an aliquot of a solution containing the test article sample was injected into a gas chromatograph and the purity of the test article sample was expressed as a percentage (area %). The test article sample was further characterized by using the procedure in SOP No. ARS 325-R1 to measure the concentration (area %) of other brominated impurities. The stability of the test article will be determined by comparing the decabromodiphenyl oxide purity (area %) of a study day-zero sample with the decabromodiphenyl oxide purity of an end-of-study sample. Stability of the test article will be confirmed if the decabromodiphenyl oxide purity (area %) of the day-zero and the end-of-study samples do not differ by more than 5 %. Chain of Custody and Sample Handling will be conducted according to established standard operating procedures.

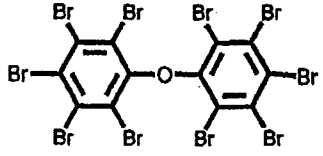
VII. Results:

The attached Conclusions and Test Article Analytical Data contains all of the test results on the test article. The identity of the test article was confirmed by Fourier Transform Infrared Spectroscopy. The purity of the test article was determined to be 97.90 area%. The test article contained three measurable

		impurities in concentrations of 0.02, 0.24 and 1.84 area %. There were no circumstances that may have affected the quality or integrity of the data.
VIII.	Regulatory Requirements:	The study conformed to the requirements of EPA TSCA (40 CFR Part 792) Good Laboratory Practice Regulations and the OECD [C(97)186/Final] Good Laboratory Practice Regulations.
IX.	Data/Record Retention:	All original raw data records shall be kept filed in the custody of the Study Chemist until the toxicology studies are completed, after which time they will be forwarded to the GLP Coordinator and stored in the designated Health and Environment archives at Albemarle Corporation, Health and Environment Department, 451 Florida Street, Baton Rouge, LA 70801.
X.	Protocol Signatures:	Paul F. Ranken Paul F. Ranken, Ph. D. STUDY CHEMIST March 13, 2001 DATE

TAB 11

CONCLUSIONS AND TEST ARTICLE ANALYTICAL DATA

Chemical Name: **CAS Number:** **Molecular Weight:** **Physical Form:** **Chemical Structure:**	Decabromodiphenyl Oxide 1163-19-5 959.05 White Powder 			
ANALYSIS	**RESULTS**		**ANALYSIS DATE**	**ANALYST**
GC	**Component** / **Area %** Decabromodiphenyl Oxide 97.90 Other Brominated Diphenyl Oxide 0.02 Other Brominated Diphenyl Oxide 0.24 Other Brominated Diphenyl Oxide 1.84		02/14/01	P.E. Smith
FT-IR	The FT-IR spectrum was obtained and it was consistent with the Aldrich standard reference spectrum of pentabromophenyl ether (decabromodiphenyl oxide). All spectra are on file with the original data.		02/14/01	W. T. Cobb
Conclusion: Based on these analytical data, the test article was identified as Decabromodiphenyl Oxide. The test article was 97.9% purity and contained three measurable impurities.				

Conclusions and Test Article Data. 2.

Characterization of Test Article by GC (Area %)

	Area %
Decabromodiphenyl Oxide	97.90
Other Brominated Diphenyl Oxide	1.84
Other Brominated Diphenyl Oxide	0.24
Other Brominated Diphenyl Oxide	0.02

Appendix 4

Analysis of Decabromodiphenyl Oxide (DBDPO) in Soil Samples From a Seedling Emergence Test

Analytical Method

The method used for the analysis of Decabromodiphenyl oxide (DBDPO) in soil samples was developed by Wildlife International, Ltd. The analytical method consisted of mixing the soil samples in a blender and on a shaker table until homogenous and weighing triplicate samples from each homogenate. The soil samples were extracted two times with tetrahydrofuran and subsequently diluted with 50% tetrahydrofuran : 50% water.

Concentrations of DBDPO were determined by high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) equipped with a Hewlett-Packard Model 1100 Variable Wavelength Detector. Chromatographic separations were achieved using a Zorbax phenyl analytical column (250 x 4.6 mm, 5 µm particle size). The instrument parameters are summarized in Table 1 and a method flow chart is provided in Figure 1.

Calibration Curves

Calibration standards containing DBDPO ranging from 1.00 to 10.0 mg/L were prepared in 50% tetrahydrofuran:50% water and analyzed with the sample set. A linear regression analysis was generated using the peak area responses versus the respective concentrations of the calibration standards. A representative calibration curve for DBDPO is presented in Figure 2. The concentration of DBDPO in the samples was determined by substituting the area peak responses into the applicable linear regression equation. Representative chromatograms of low and high calibration standards for DBDPO are presented in Figures 3 and 4, respectively.

Example Calculations

A sample calculation of sample number 439-101-5 having a nominal concentration of 781 µg/g DBDPO in the spray mixture follows:

Initial mass (M_1) : 10.0 grams
Initial final volume (V_1): 200 mL
Secondary dilution (V_2): 1.00 → 6.00 mL
Dilution Factor (V_1/M_1) x (V_2) = 120
Peak Area: 1117.61475
Linear regression equation:

Slope: 201.44
$Y_{Intercept}$: -2.9346

$$\text{Concentration DBDPO } (\mu g/g) = \frac{(\text{Peak area} - Y_{intercept}) \times \text{Dilution factor}}{\text{Slope}}$$

$$\text{Concentration DBDPO } (\mu g/g) = \frac{(1117.61475 + 2.9346) \times 120}{201.44}$$

$$\text{Concentration DBDPO } (\mu g/g) = 668\ \mu g/g$$

$$\text{Percent of Nominal Conc.} = \frac{668\ \mu g/g}{781\ \mu g/g} \times 100$$

$$\text{Percent of Nominal Conc.} = 85.5\%$$

RESULTS

Sample Analysis

Soil samples were collected from a study designed to determine the effects of DBDPO on the seedling emergence of non-target plants. Samples were processed and analyzed between May 23 and 25, 2001. Concentrations of DBDPO in soil in the range of 391 to 6250 µg/g yielded percent recoveries from 52.2 to 101%. The mean percent recoveries of triplicate samples at 391, 781, 1563, 3125 and 6250 µg/g were 74.8, 90.5, 75.3, 67.1 and 85.6%, respectively. Quality control samples fortified at 300, 1000 and 6500 µg/g yielded percent recoveries of 84.8, 84.5 and 99.6%, respectively. The control sample was devoid of DBDPO. Analytical results for all exposure and quality control samples are presented in Table 2. A chromatogram of a control sample (439-101-1) is presented in Figure 5. A representative chromatogram of a soil extract (439-101-2) is presented in Figure 6.

Table 1

Typical HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Hewlett-Packard Model 1100 Variable Wavelength Detector			
ANALYTICAL COLUMN:	Zorbax phenyl (250 mm x 4.6 mm, 5 µm particle size)			
FLOW RATE:	1.00 mL/min			
OVEN TEMPERATURE:	40°C			
MOBILE PHASE:	Solvent A: 0.1% H_3PO_4 Solvent B: CH_3CN			
GRADIENT:	Time	% A	%B	Flow (mL/min)
	0.01	30.0	70.0	1.00
	2.00	30.0	70.0	1.00
	10.0	2.00	98.0	1.00
	16.0	2.00	98.0	1.00
	16.1	30.0	70.0	1.00
	20.0	30.0	70.0	1.00
INJECTION VOLUME:	50 µL			
DBDPO RETENTION TIME:	Approximately 13.3 minutes			
PRIMARY ANALYTICAL WAVELENGTH:	220 nm			

Table 2

Measured Concentration of DBDPO in Samples from a Seedling Emergence Study

Nominal Test Concentration (µg/g)	Sample Number (439-101)[1]	Measured Concentration (µg/g)[2,3]	Percent of Nominal[2] (%)	Mean Concentration (µg/g)	Mean Percent of Nominal (%)
0.0	MAB-2	< LOQ	--	--	--
300	MAS-4	255	84.8	--	--
1000	MAS-5	845	84.5	--	--
6500	MAS-6	6472	99.6	--	--
Negative Control	1	< LOQ	--	--	--
391	2	271	69.3	292	74.8
	3	284	72.6		
	4	322	82.3		
781	5	668	85.5	707	90.5
	6	667	85.4		
	7	785	101		
1563	8	1224	78.3	1177	75.3
	9	928	59.4		
	10	1377	88.1		
3125	11	1630	52.2	2098	67.1
	12	2465	78.9		
	13	2198	70.3		
6250	14	5423	86.8	5349	85.6
	15	5318	85.1		
	16	5306	84.9		

[1] MAB refers to an unfortified matrix blank. MAS refers to a fortified quality control sample.

[2] The limit of quantitation (LOQ) was defined as 100 µg/g, calculated as the product of the lowest calibration standard (1.00 mg/L) and the dilution factor of the matrix blank sample (100).

[3] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

METHOD OUTLINE FOR THE PROCESSING OF DBDPO IN SOIL

Pre-rinse all glassware with tetrahydrofuran (THF).

↓

Prepare recovery samples by fortifying 10.0 grams of soil (contained in 8-oz. French square bottles) with the appropriate DBDPO stock solution. For test samples, homogenize each sample in a blender for approximately 2 minutes, stopping at 30-second intervals to stir the sample. Transfer the entire contents to a French square bottle, secure on a shaker table and shake for approximately 30 minutes at a setting of 300 rpm. From each sample, transfer 10.0 grams of mixed soil to an 8-oz. French square bottle.

↓

Add 100 mL of THF to each test and QC sample. Seal the samples and secure to a shaker table; shake at 250 rpm for approximately 15 minutes.

↓

Centrifuge the samples for approximately 5 minutes at a setting of 1500 rpm.

↓

Pour the supernatant through a plug of glass wool (contained in a funnel) and collect the extract in a 200-mL volumetric flask.

↓

Add an additional 90 mL of THF to the French square bottles. Repeat the extraction procedure, combining the extract in the volumetric flask. Adjust the flask to volume with THF.

↓

Prepare secondary dilutions of all samples using 50% THF: 50% H_2O (v:v).

↓

Transfer the diluted extract to an autosampler vial and submit for HPLC/UV analysis.

Figure 1. A method flowchart for the analysis of DBDPO in soil samples.

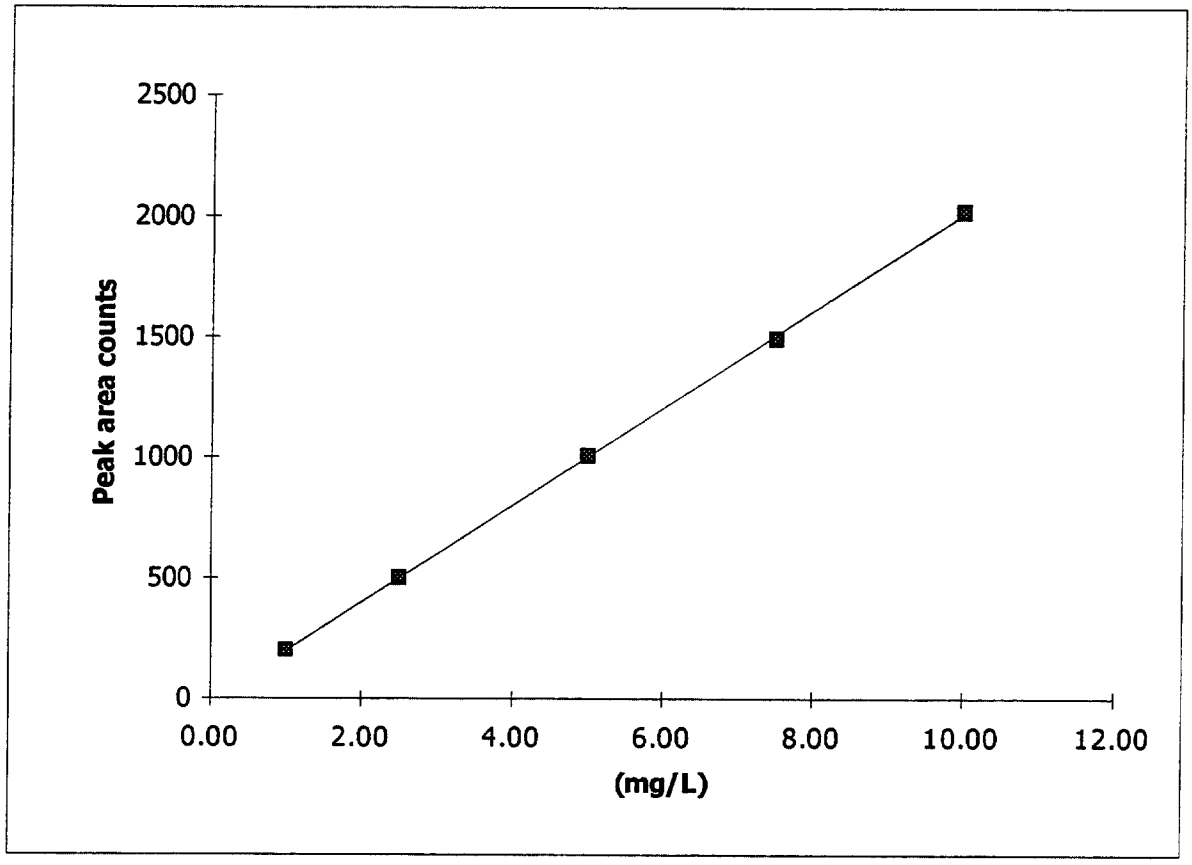


Figure 2. A representative calibration curve for DBDPO. Slope = 201.44; Y-Intercept = -2.9346; $r^2 = 0.9999$.

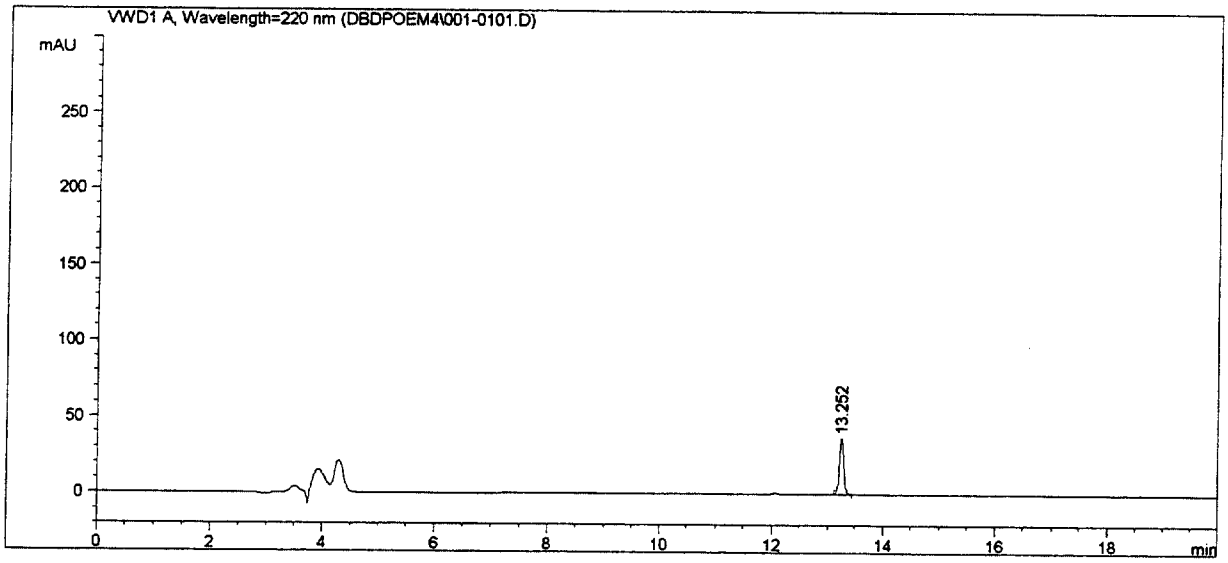


Figure 3. A representative chromatogram of a 1.00 mg/L calibration standard.

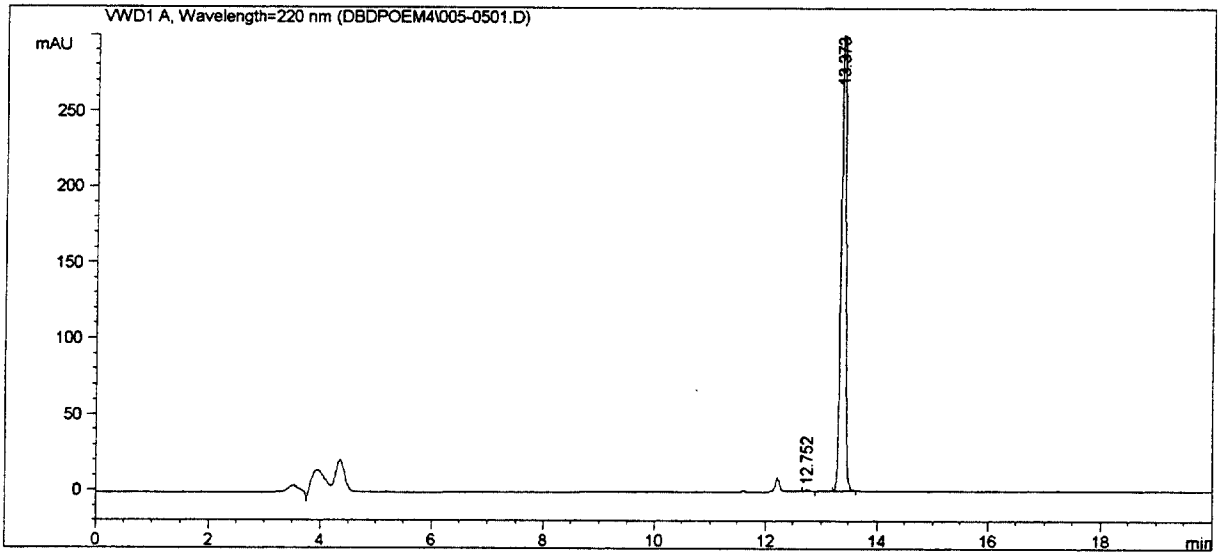


Figure 4. A representative chromatogram of a 10.0 mg/L calibration standard.

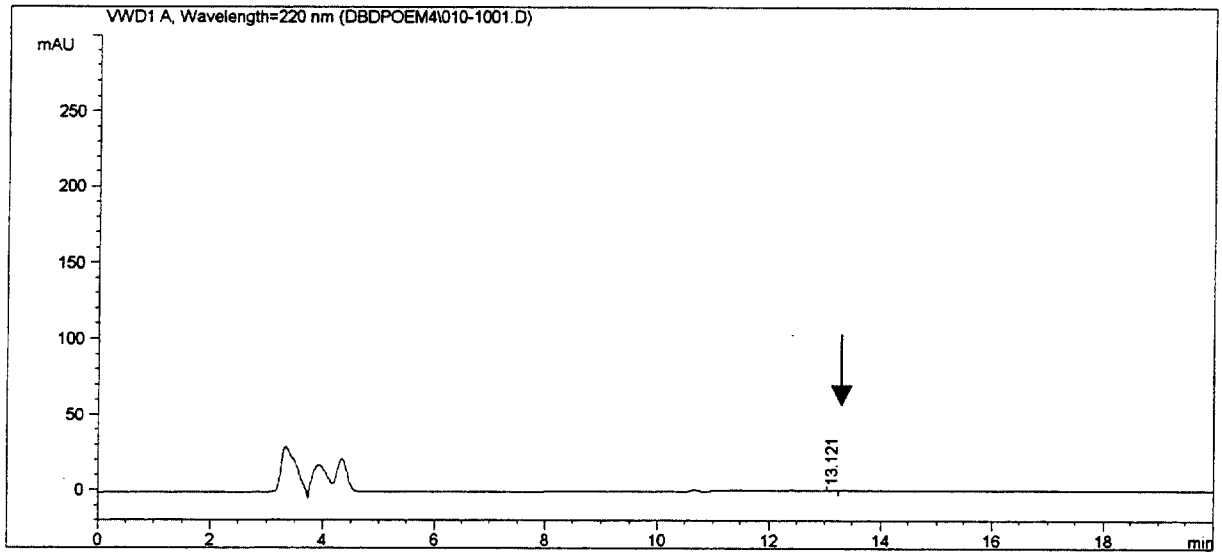


Figure 5. A representative chromatogram of a control sample (439-101-1). The arrow indicates the retention time of DBDPO.

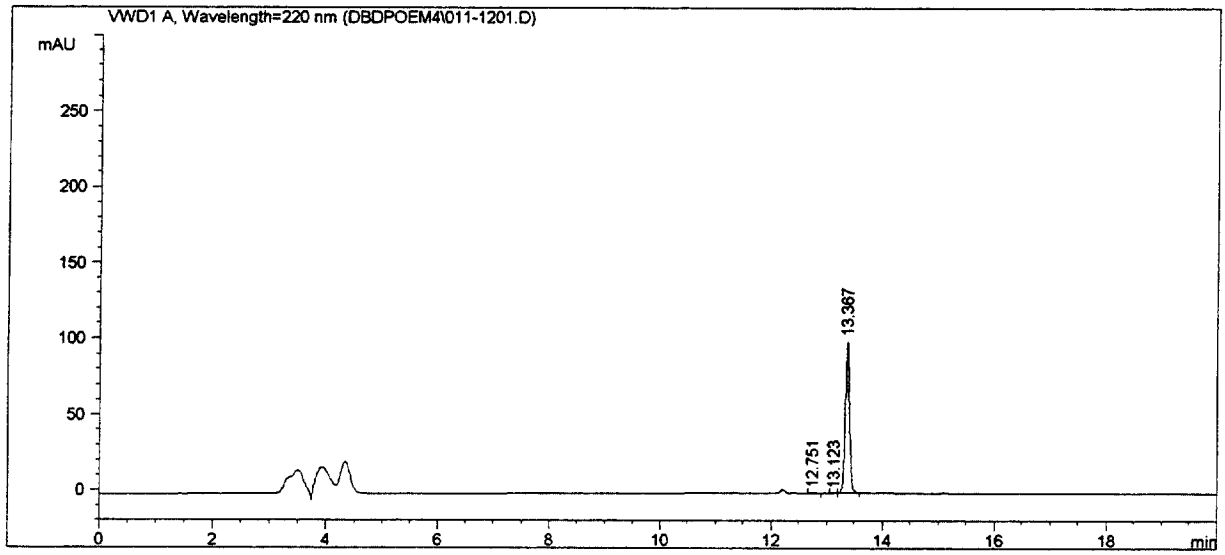


Figure 6. A representative chromatogram of a soil extract (439-101-2, 391 µg/g nominal concentration).

Appendix 5

Environmental Conditions

Date	Temperature (°C)			Relative Humidity (%)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
3/28/01[1]	21	30	25	10	36	19
3/29/01	21	27	24	23	54	33
3/30/01	18	27	23	33	67	50
3/31/01	18	26	21	26	55	40
4/01/01	18	26	21	26	49	38
4/02/01	18	27	22	19	45	32
4/03/01[1]	18	28	22	15	49	30
4/04/01[1]	18	27	22	15	47	31
4/05/01	18	30	23	14	42	32
4/06/01	18	27	22	29	69	44
4/07/01[1]	18	26	22	32	74	50
4/08/01	18	27	22	35	71	49
4/09/01	18	33	25	44	77	58
4/10/01[2]	18	27	22	36	79	55
4/11/01	18	25	22	49	75	59
4/12/01	18	32	24	48	79	64
4/13/01	19	30	24	31	78	55
4/14/01[1]	18	28	23	24	53	40
4/15/01	18	28	23	23	64	45
4/16/01[2]	18	26	22	31	63	49
4/17/01[1]	18	26	22	18	58	33
4/18/01	18	26	22	14	42	26

[1] Indicates days on which all species were watered.
[2] Indicates days on which only cucumber, soybean and corn were filled.

Appendix 6.1

Corn Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	9	*4*	9.75	0.50
391 mg/kg	10	10	9	10	*4*	9.75	0.50
781 mg/kg	10	10	10	9	*4*	9.75	0.50
1563 mg/kg	10	10	10	9	*4*	9.75	0.50
3125 mg/kg	10	10	9	9	*4*	9.50	0.58
6250 mg/kg	9	10	10	10	*4*	9.75	0.50

Day 14

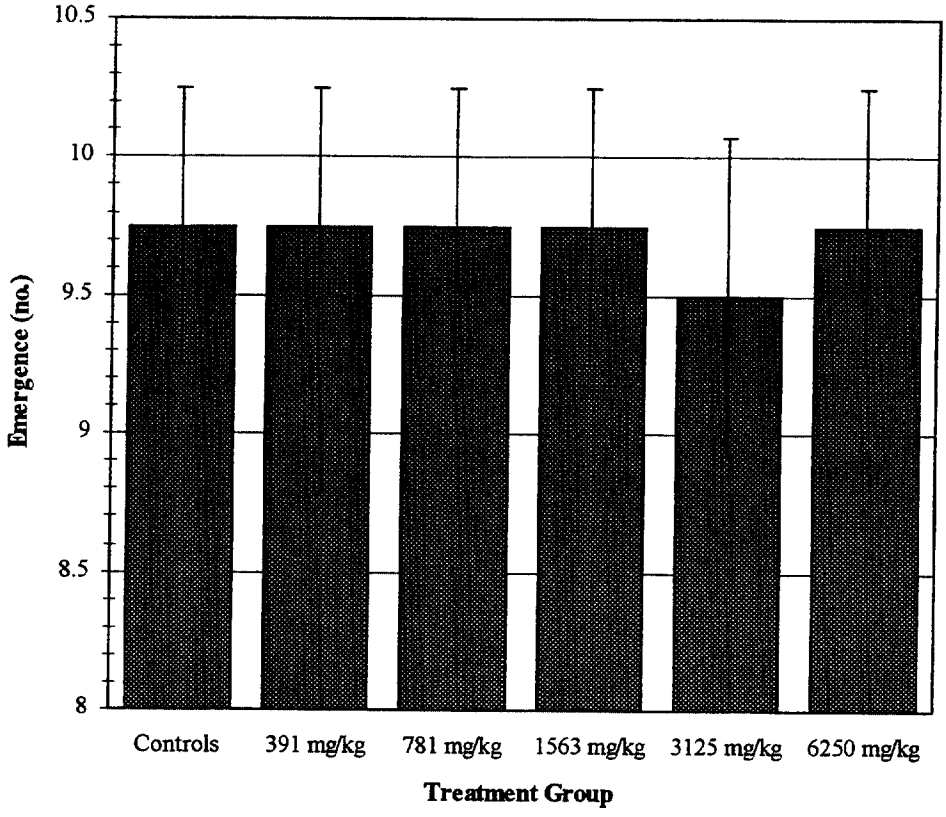
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	9	*4*	9.75	0.50
391 mg/kg	10	10	9	10	*4*	9.75	0.50
781 mg/kg	10	10	10	9	*4*	9.75	0.50
1563 mg/kg	10	10	10	9	*4*	9.75	0.50
3125 mg/kg	10	10	9	9	*4*	9.50	0.58
6250 mg/kg	9	10	10	10	*4*	9.75	0.50

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	9	*4*	9.75	0.50
391 mg/kg	10	10	9	10	*4*	9.75	0.50
781 mg/kg	10	10	10	9	*4*	9.75	0.50
1563 mg/kg	10	10	10	9	*4*	9.75	0.50
3125 mg/kg	10	10	9	9	*4*	9.50	0.58
6250 mg/kg	9	10	10	10	*4*	9.75	0.50

Appendix 6.2

Mean Corn Emergence on Day 21



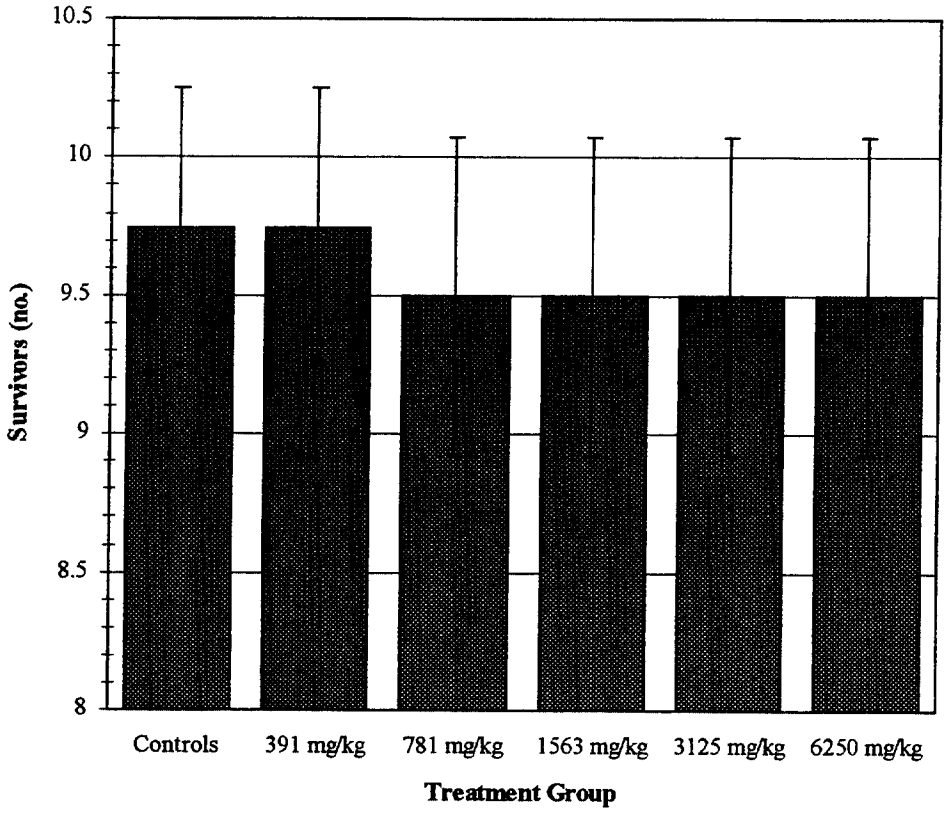
Appendix 6.3

Corn 21-Day Survival

	Day 21						
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	9	*4*	9.75	0.50
391 mg/kg	10	10	9	10	*4*	9.75	0.50
781 mg/kg	10	9	10	9	*4*	9.50	0.58
1563 mg/kg	10	9	10	9	*4*	9.50	0.58
3125 mg/kg	10	10	9	9	*4*	9.50	0.58
6250 mg/kg	9	10	9	10	*4*	9.50	0.58

Appendix 6.4

Mean Corn 21-Day Survival



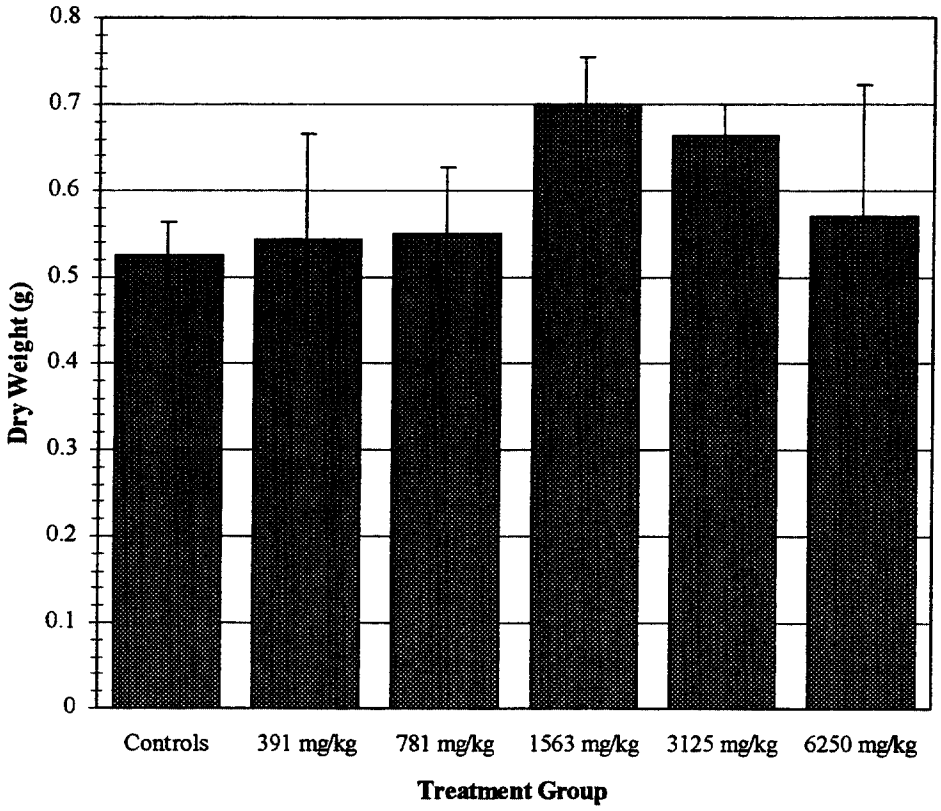
Appendix 6.5

Corn Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.4841	0.5068	0.5339	0.5745	*4*	0.5248	0.03887
391 mg/kg	0.6055	0.6441	0.3664	0.5574	*4*	0.5433	0.12317
781 mg/kg	0.6158	0.4613	0.5136	0.6122	*4*	0.5507	0.07614
1563 mg/kg	0.7476	0.6980	0.6239	0.7282	*4*	0.6994	0.05432
3125 mg/kg	0.6193	0.6640	0.7072	0.6644	*4*	0.6637	0.03587
6250 mg/kg	0.5225	0.3761	0.6876	0.6966	*4*	0.5707	0.15241

Appendix 6.6

Mean Corn Dry Weight



Appendix 6.7

Corn Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	44	49	45	41	44	43	43	41	30	50	*10*	43.0	5.46
	B	50	4	46	51	43	49	53	26	50	50	*10*	42.2	15.49
	C	45	46	52	52	49	51	39	49	40	49	*10*	47.2	4.66
	D	.	27	56	61	51	13	53	57	54	49	*9*	46.8	15.96
391 mg/kg	A	50	48	42	48	50	51	55	31	44	54	*10*	47.3	6.98
	B	40	52	56	56	54	51	43	54	37	53	*10*	49.6	6.95
	C	.	43	35	32	43	49	44	48	39	22	*9*	39.4	8.59
	D	48	55	57	53	53	35	38	58	47	47	*10*	49.1	7.74
781 mg/kg	A	51	42	58	54	62	45	43	55	47	35	*10*	49.2	8.27
	B	10	16	29	45	42	44	40	45	45	.	*9*	35.1	13.59
	C	34	48	40	41	52	47	47	48	46	39	*10*	44.2	5.45
	D	.	53	53	55	48	43	39	44	54	52	*9*	49.0	5.74
1563 mg/kg	A	48	52	52	53	70	33	56	66	58	63	*10*	55.1	10.41
	B	58	59	46	57	52	52	59	60	66	.	*9*	56.6	5.79
	C	44	48	57	62	52	60	51	47	46	50	*10*	51.7	6.09
	D	.	59	55	46	44	51	49	51	62	37	*9*	50.4	7.68
3125 mg/kg	A	47	42	44	52	50	42	50	55	46	41	*10*	46.9	4.75
	B	54	49	53	53	49	48	50	49	53	49	*10*	50.7	2.26
	C	.	53	54	50	39	56	48	51	58	58	*9*	51.9	5.95
	D	.	32	56	33	60	57	53	59	54	55	*9*	51.0	10.72
6250 mg/kg	A	.	50	47	4	44	52	49	44	41	47	*9*	42.0	14.65
	B	40	36	42	37	30	39	44	40	45	40	*10*	39.3	4.30
	C	44	49	46	58	57	59	67	56	57	.	*9*	54.8	7.21
	D	47	38	45	56	45	51	60	54	53	52	*10*	50.1	6.40

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

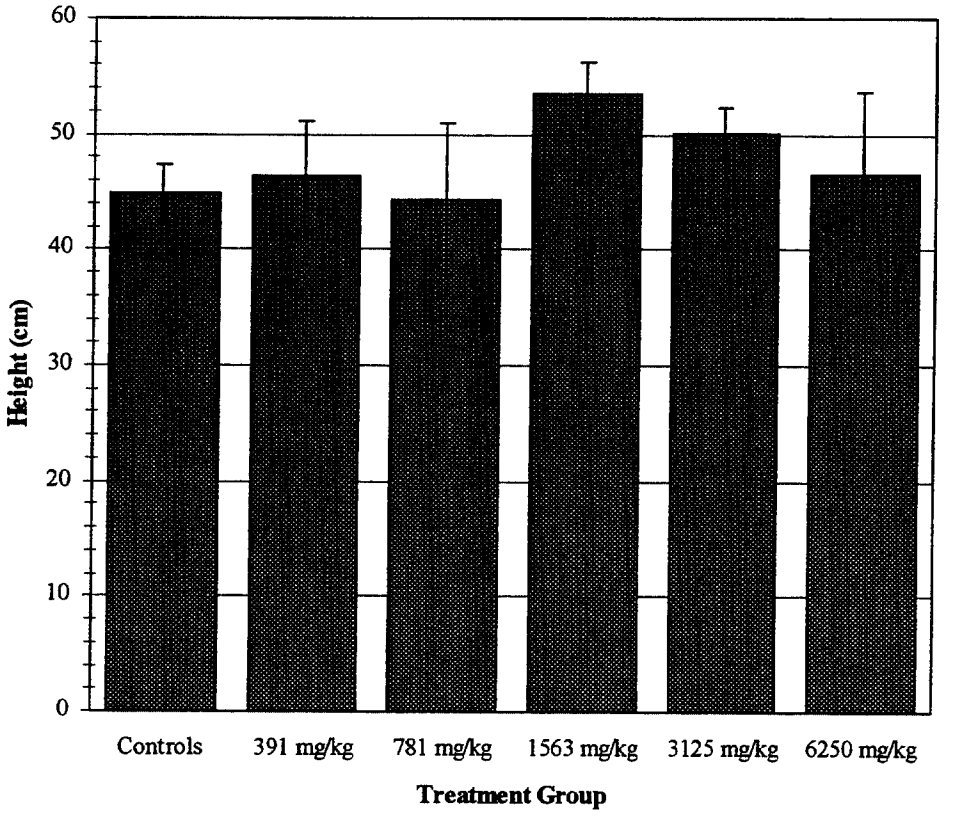
Appendix 6.8

Corn Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	43.0	42.2	47.2	46.8	*4*	44.8	2.56
391 mg/kg	47.3	49.6	39.4	49.1	*4*	46.4	4.72
781 mg/kg	49.2	35.1	44.2	49.0	*4*	44.4	6.60
1563 mg/kg	55.1	56.6	51.7	50.4	*4*	53.5	2.86
3125 mg/kg	46.9	50.7	51.9	51.0	*4*	50.1	2.21
6250 mg/kg	42.0	39.3	54.8	50.1	*4*	46.5	7.15

Appendix 6.9

Mean Corn Height on Day 21



Appendix 6.10

Corn Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	30.LC	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	3	9.5
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	50.N	0.-	0.-	0.-	0.-	*9*	6	16.7
391 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
781 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
1563 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
3125 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
6250 mg/kg	A	.	0.-	0.-	50.N	0.-	0.-	0.-	0.-	0.-	0.-	*9*	6	16.7
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

Appendix 7.1

Cucumber Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	10	9	10	*4*	9.25	0.96
391 mg/kg	10	10	10	9	*4*	9.75	0.50
781 mg/kg	10	10	9	10	*4*	9.75	0.50
1563 mg/kg	10	10	10	10	*4*	10.00	0.00
3125 mg/kg	10	9	10	10	*4*	9.75	0.50
6250 mg/kg	10	10	10	0	*4*	7.50	5.00

Day 14

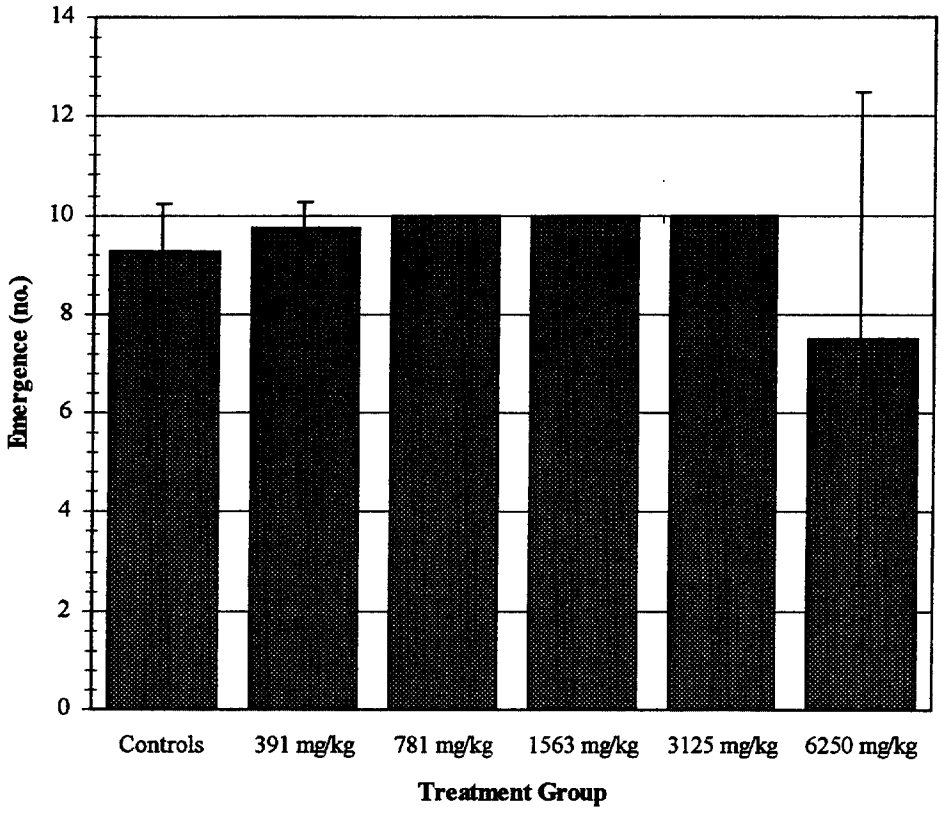
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	10	9	10	*4*	9.25	0.96
391 mg/kg	10	10	10	9	*4*	9.75	0.50
781 mg/kg	10	10	9	10	*4*	9.75	0.50
1563 mg/kg	10	10	10	10	*4*	10.00	0.00
3125 mg/kg	10	10	10	10	*4*	10.00	0.00
6250 mg/kg	10	10	10	0	*4*	7.50	5.00

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	10	9	10	*4*	9.25	0.96
391 mg/kg	10	10	10	9	*4*	9.75	0.50
781 mg/kg	10	10	10	10	*4*	10.00	0.00
1563 mg/kg	10	10	10	10	*4*	10.00	0.00
3125 mg/kg	10	10	10	10	*4*	10.00	0.00
6250 mg/kg	10	10	10	0	*4*	7.50	5.00

Appendix 7.2

Mean Cucumber Emergence on Day 21



Appendix 7.3

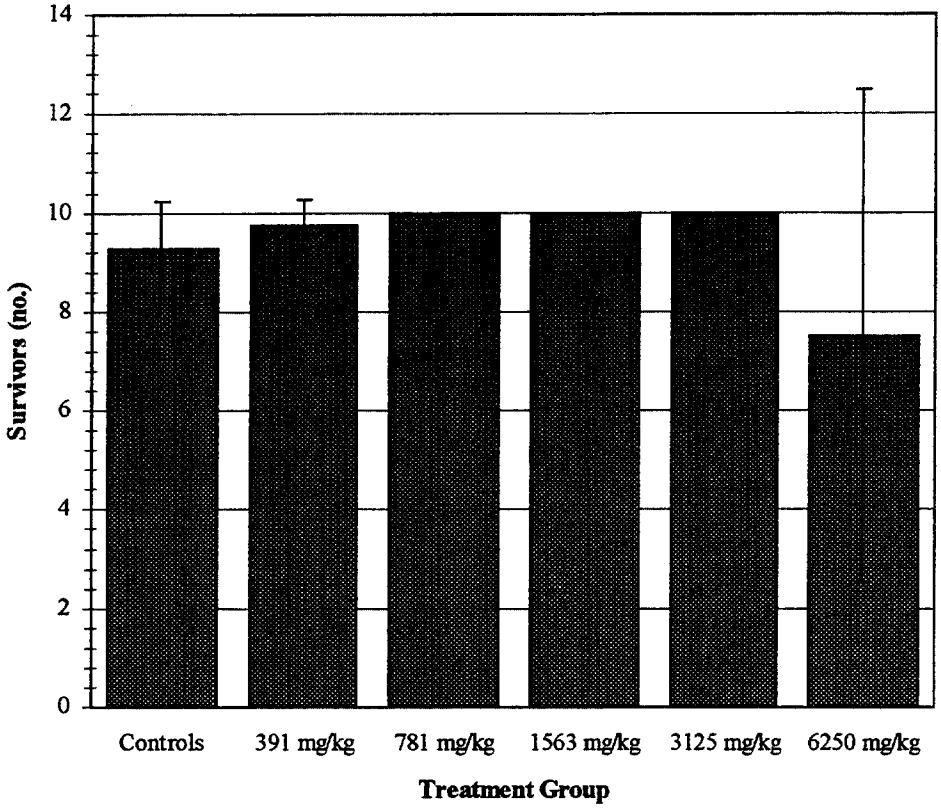
Cucumber 21-Day Survival

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	10	9	10	*4*	9.25	0.96
391 mg/kg	10	10	10	9	*4*	9.75	0.50
781 mg/kg	10	10	10	10	*4*	10.00	0.00
1563 mg/kg	10	10	10	10	*4*	10.00	0.00
3125 mg/kg	10	10	10	10	*4*	10.00	0.00
6250 mg/kg	10	10	10	0	*4*	7.50	5.00

Appendix 7.4

Mean Cucumber 21-Day Survival



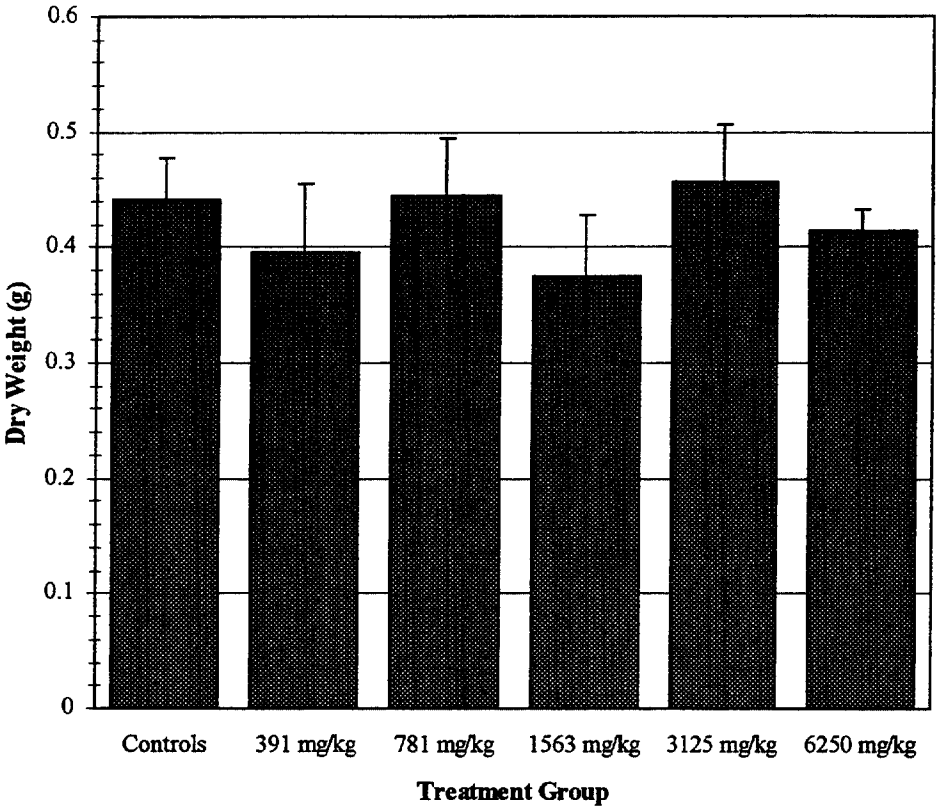
Appendix 7.5

Cucumber Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.4178	0.4400	0.4922	0.4190	*4*	0.4423	0.03481
391 mg/kg	0.3320	0.3565	0.4428	0.4503	*4*	0.3954	0.06002
781 mg/kg	0.4337	0.4528	0.3864	0.5065	*4*	0.4448	0.04968
1563 mg/kg	0.4453	0.3454	0.3846	0.3269	*4*	0.3756	0.05233
3125 mg/kg	0.3989	0.4753	0.4355	0.5150	*4*	0.4562	0.05008
6250 mg/kg	0.3956	0.4322	0.4154	.	*3*	0.4144	0.01831

Appendix 7.6

Mean Cucumber Dry Weight



Appendix 7.7

Cucumber Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	.	9	8	13	12	10	13	10	13	*8*	11.0	2.00
	B	18	19	15	15	15	22	14	15	13	13	*10*	15.9	2.88
	C	.	16	18	20	16	19	15	18	15	16	*9*	17.0	1.80
	D	13	14	10	15	15	14	15	13	15	12	*10*	13.6	1.65
391 mg/kg	A	9	10	12	14	11	5	13	14	9	11	*10*	10.8	2.74
	B	13	14	11	13	13	12	11	13	10	10	*10*	12.0	1.41
	C	18	19	19	18	16	21	15	21	19	15	*10*	18.1	2.18
	D	.	10	13	14	15	14	13	8	14	12	*9*	12.6	2.24
781 mg/kg	A	13	16	12	16	16	14	14	9	13	13	*10*	13.6	2.17
	B	12	20	11	17	18	17	16	19	20	18	*10*	16.8	3.08
	C	18	17	17	12	13	18	18	0.25	16	15	*10*	14.4	5.41
	D	13	18	11	17	15	18	19	17	16	21	*10*	16.5	2.92
1563 mg/kg	A	15	15	12	14	16	16	17	6	15	13	*10*	13.9	3.14
	B	15	14	12	14	16	15	14	16	14	13	*10*	14.3	1.25
	C	11	12	13	15	13	15	13	15	14	12	*10*	13.3	1.42
	D	12	11	11	9	13	14	15	14	11	13	*10*	12.3	1.83
3125 mg/kg	A	13	14	15	13	17	13	13	14	12	11	*10*	13.5	1.65
	B	14	15	17	18	19	19	18	1	15	18	*10*	15.4	5.36
	C	20	21	20	18	19	17	16	18	15	17	*10*	18.1	1.91
	D	18	20	17	14	15	18	17	15	17	13	*10*	16.4	2.12
6250 mg/kg	A	9	14	12	16	14	10	12	12	16	16	*10*	13.1	2.51
	B	11	16	15	17	19	17	15	18	18	17	*10*	16.3	2.26
	C	15	14	17	13	13	15	17	18	18	17	*10*	15.7	1.95
	D	.	.	.	.	.	.	.	.	.	.	*0*	.	.

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

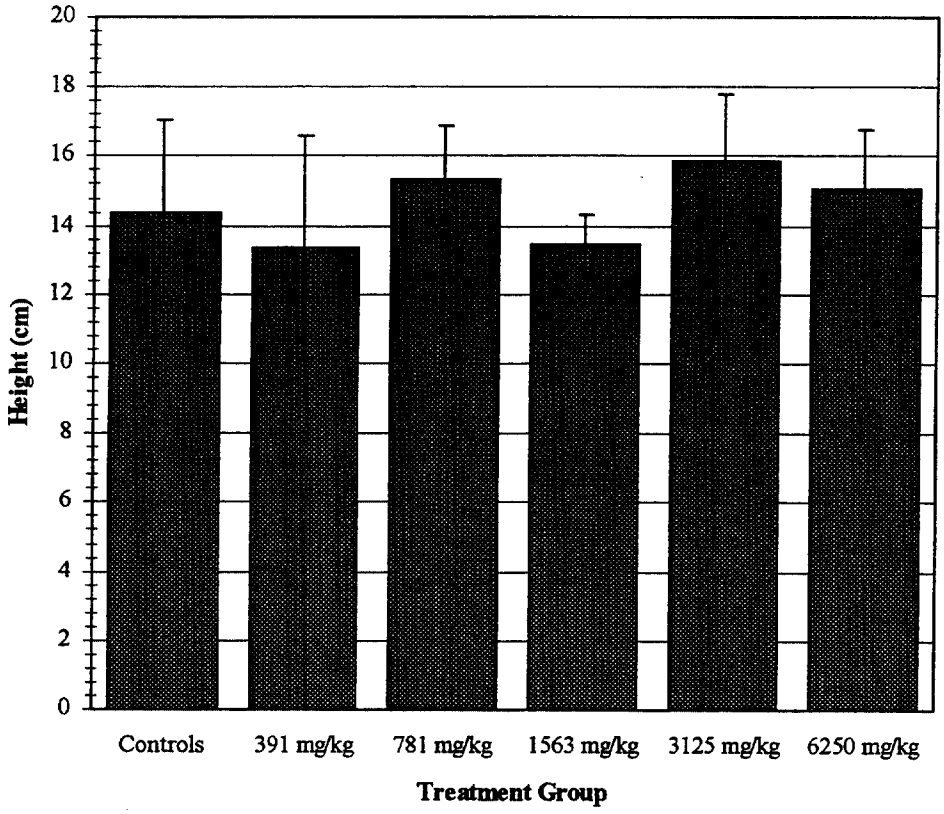
Appendix 7.8

Cucumber Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	11.0	15.9	17.0	13.6	*4*	14.4	2.66
391 mg/kg	10.8	12.0	18.1	12.6	*4*	13.4	3.24
781 mg/kg	13.6	16.8	14.4	16.5	*4*	15.3	1.56
1563 mg/kg	13.9	14.3	13.3	12.3	*4*	13.5	0.87
3125 mg/kg	13.5	15.4	18.1	16.4	*4*	15.9	1.92
6250 mg/kg	13.1	16.3	15.7	.	*3*	15.0	1.70

Appendix 7.9

Mean Cucumber Height on Day 21



Appendix 7.10

Cucumber Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
391 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
781 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
1563 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
3125 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
6250 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	.	.	.	.	.	.	.	.	.	*0*	.	.

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

Appendix 8.1

Onion Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	4	9	8	6	*4*	6.75	2.22
391 mg/kg	9	3	5	9	*4*	6.50	3.00
781 mg/kg	6	8	5	3	*4*	5.50	2.08
1563 mg/kg	10	9	8	6	*4*	8.25	1.71
3125 mg/kg	8	7	10	9	*4*	8.50	1.29
6250 mg/kg	9	8	9	9	*4*	8.75	0.50

Day 14

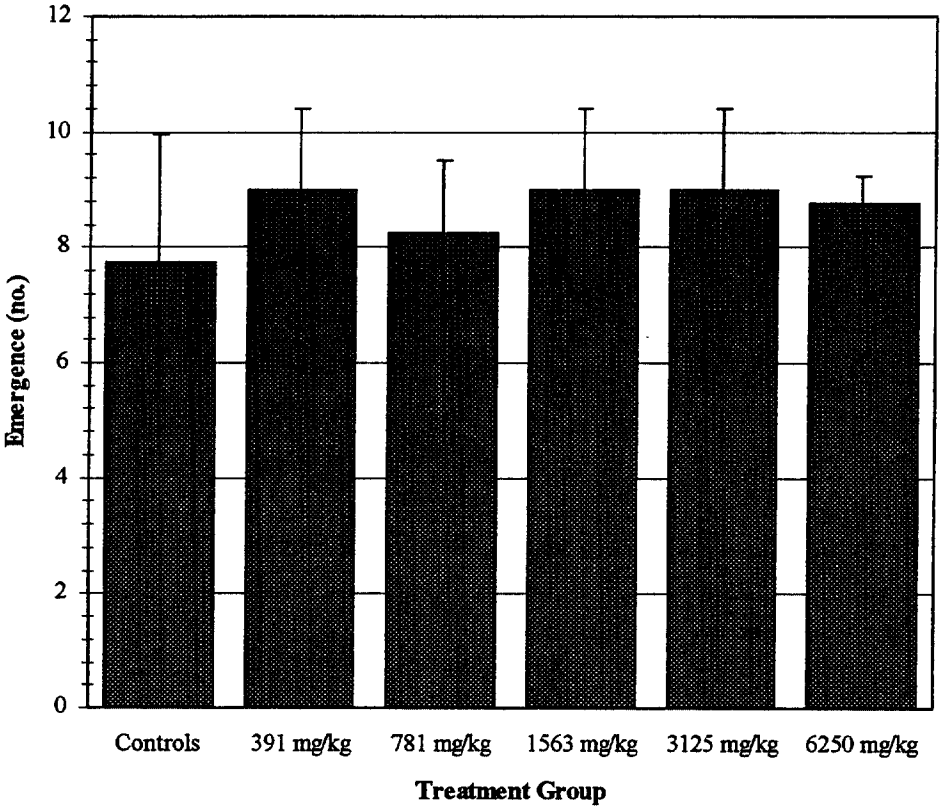
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	5	9	10	7	*4*	7.75	2.22
391 mg/kg	9	7	10	10	*4*	9.00	1.41
781 mg/kg	10	8	8	8	*4*	8.50	1.00
1563 mg/kg	10	10	9	7	*4*	9.00	1.41
3125 mg/kg	9	7	10	10	*4*	9.00	1.41
6250 mg/kg	9	8	9	9	*4*	8.75	0.50

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	5	9	10	7	*4*	7.75	2.22
391 mg/kg	9	7	10	10	*4*	9.00	1.41
781 mg/kg	10	7	8	8	*4*	8.25	1.26
1563 mg/kg	10	10	9	7	*4*	9.00	1.41
3125 mg/kg	9	7	10	10	*4*	9.00	1.41
6250 mg/kg	9	8	9	9	*4*	8.75	0.50

Appendix 8.2

Mean Onion Emergence on Day 21



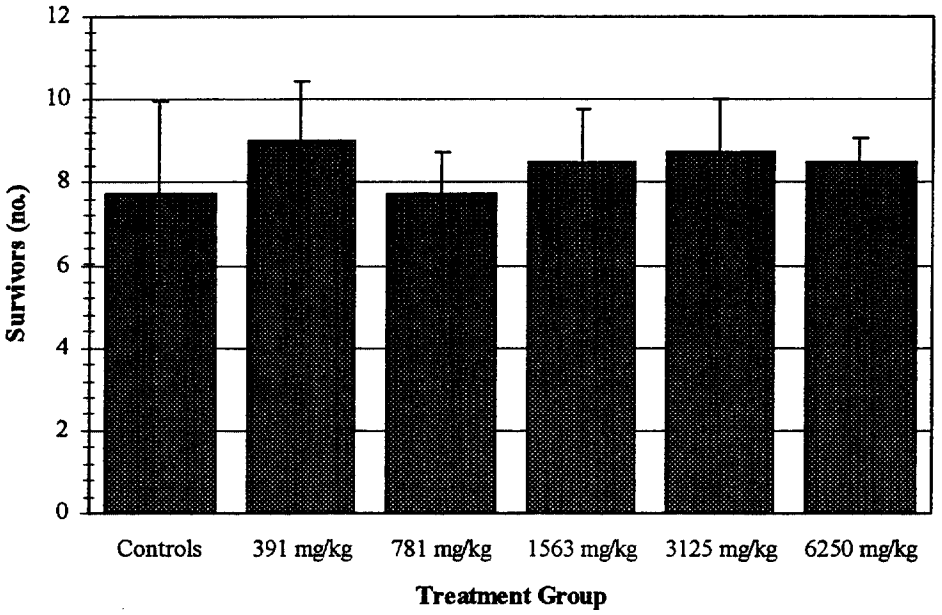
Appendix 8.3

Onion 21-Day Survival

Treatment Group	Day 21 Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	5	9	10	7	*4*	7.75	2.22
391 mg/kg	9	7	10	10	*4*	9.00	1.41
781 mg/kg	9	7	7	8	*4*	7.75	0.96
1563 mg/kg	9	10	8	7	*4*	8.50	1.29
3125 mg/kg	9	7	10	9	*4*	8.75	1.26
6250 mg/kg	8	8	9	9	*4*	8.50	0.58

Appendix 8.4

Mean Onion 21-Day Survival



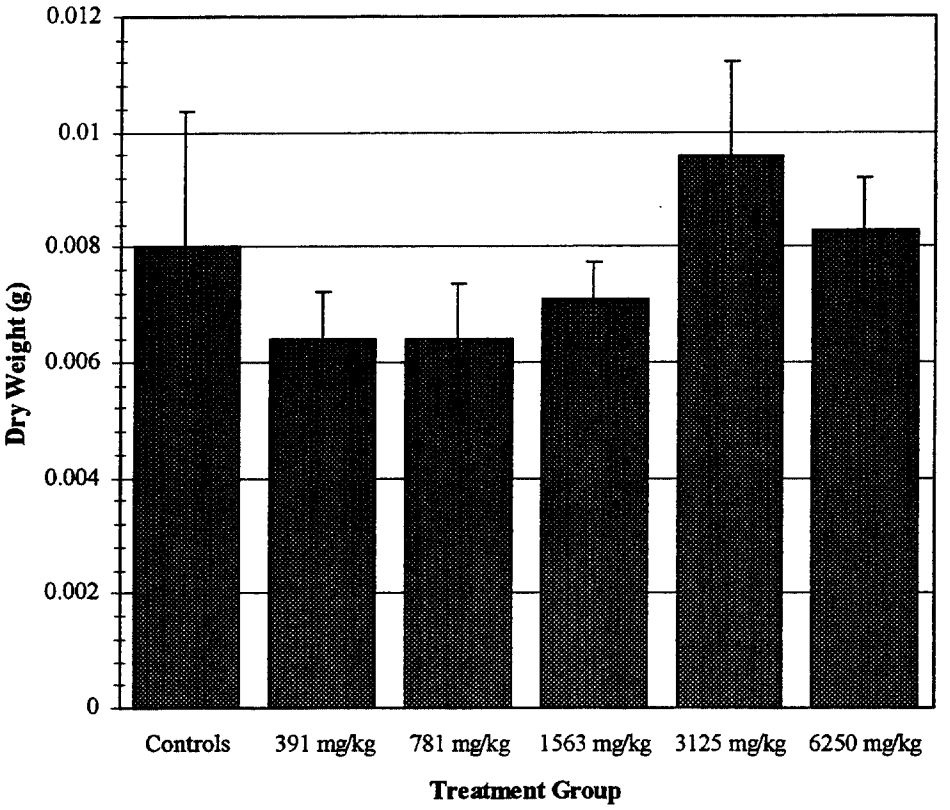
Appendix 8.5

Onion Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.00646	0.01150	0.00700	0.00710	*4*	0.00802	0.002340
391 mg/kg	0.00657	0.00684	0.00518	0.00702	*4*	0.00640	0.000836
781 mg/kg	0.00521	0.00703	0.00730	0.00610	*4*	0.00641	0.000950
1563 mg/kg	0.00709	0.00783	0.00623	0.00719	*4*	0.00708	0.000659
3125 mg/kg	0.01038	0.00754	0.01132	0.00909	*4*	0.00958	0.001639
6250 mg/kg	0.00730	0.00935	0.00777	0.00869	*4*	0.00828	0.000919

Appendix 8.6

Mean Onion Dry Weight



Appendix 8.7

Onion Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	.	.	.	.	0.25	4	9	7	9	*5*	5.9	3.74
	B	.	7	9	8	5	11	10	10	7	11	*9*	8.7	2.06
	C	1	1	6	9	6	8	7	9	12	13	*10*	7.2	3.99
	D	.	.	.	0.25	7	7	14	7	5	1	*7*	5.9	4.58
391 mg/kg	A	7	7	4	6	5	3	5	8	6	.	*9*	5.7	1.58
	B	.	.	.	6	5	10	3	4	12	1	*7*	5.9	3.89
	C	1	8	6	5	6	5	2	7	8	5	*10*	5.3	2.31
	D	7	2	6	8	7	13	8	6	6	7	*10*	7.0	2.71
781 mg/kg	A	6	12	9	2	3	1	5	6	5	.	*9*	5.4	3.43
	B	.	.	.	1	6	5	14	6	6	5	*7*	6.1	3.89
	C	.	.	2	6	10	5	8	9	8	.	*7*	6.9	2.73
	D	.	.	7	6	10	6	7	2	1	5	*8*	5.5	2.88
1563 mg/kg	A	6	6	6	5	8	6	6	7	7	.	*9*	6.3	0.87
	B	8	17	6	7	6	6	7	6	6	6	*10*	7.5	3.41
	C	.	1	9	6	6	6	6	8	8	.	*8*	6.3	2.43
	D	.	.	.	8	10	12	11	7	6	4	*7*	8.3	2.87
3125 mg/kg	A	5	7	9	8	11	5	7	7	7	.	*9*	7.3	1.87
	B	.	.	.	9	5	5	6	12	6	2	*7*	6.4	3.21
	C	11	11	11	9	10	9	10	2	9	11	*10*	9.3	2.71
	D	11	10	9	5	8	6	6	6	9	.	*9*	7.8	2.11
6250 mg/kg	A	.	10	8	6	7	13	7	9	3	.	*8*	7.9	2.95
	B	.	.	7	10	5	10	10	14	10	9	*8*	9.4	2.62
	C	.	8	10	8	6	5	11	8	6	5	*9*	7.4	2.13
	D	.	8	4	10	8	5	9	10	8	8	*9*	7.8	2.05

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

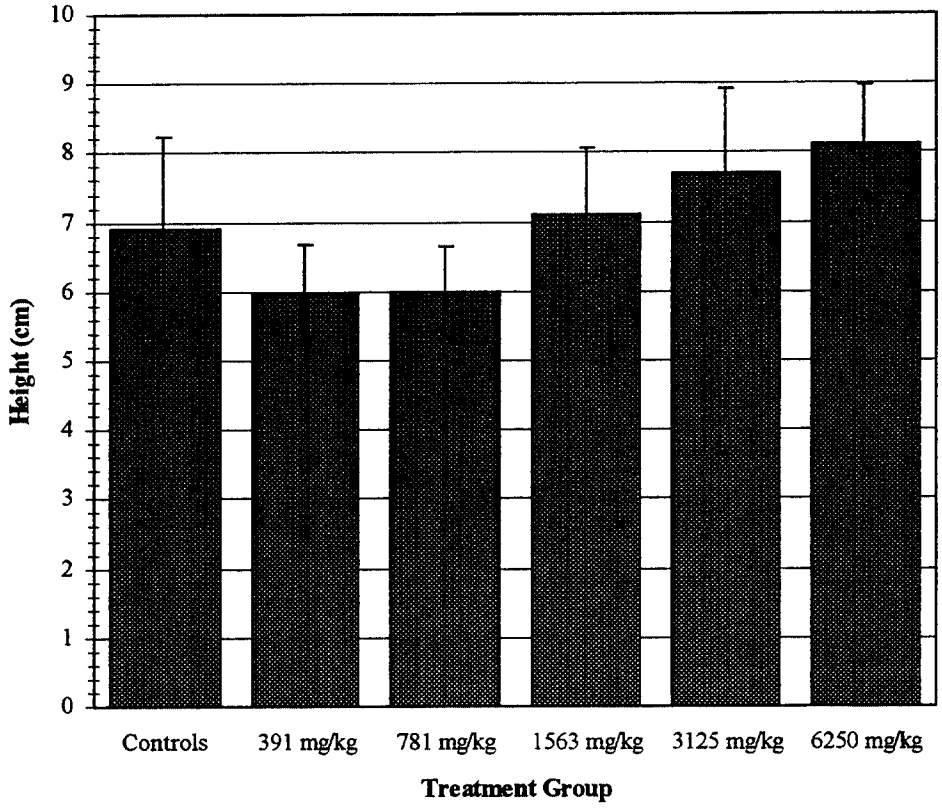
Appendix 8.8

Onion Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	5.9	8.7	7.2	5.9	*4*	6.9	1.33
391 mg/kg	5.7	5.9	5.3	7.0	*4*	6.0	0.73
781 mg/kg	5.4	6.1	6.9	5.5	*4*	6.0	0.66
1563 mg/kg	6.3	7.5	6.3	8.3	*4*	7.1	0.98
3125 mg/kg	7.3	6.4	9.3	7.8	*4*	7.7	1.20
6250 mg/kg	7.9	9.4	7.4	7.8	*4*	8.1	0.86

Appendix 8.9

Mean Onion Height on Day 21



Appendix 8.10

Onion Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	.	.	.	.	0.-	0.-	0.-	0.-	0.-	*5*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
391 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	.	*9*	0	0.0
	B	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
781 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
	B	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	C	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*8*	13	35.4
	D	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
1563 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*9*	11	33.3
	D	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
3125 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	.	*9*	0	0.0
	B	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
6250 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*9*	11	33.3
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

Appendix 9.1

RYEGRASS Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	10	8	*4*	9.00	1.15
391 mg/kg	9	9	9	10	*4*	9.25	0.50
781 mg/kg	8	9	10	10	*4*	9.25	0.96
1563 mg/kg	9	7	8	10	*4*	8.50	1.29
3125 mg/kg	10	10	9	9	*4*	9.50	0.58
6250 mg/kg	10	9	10	10	*4*	9.75	0.50

Day 14

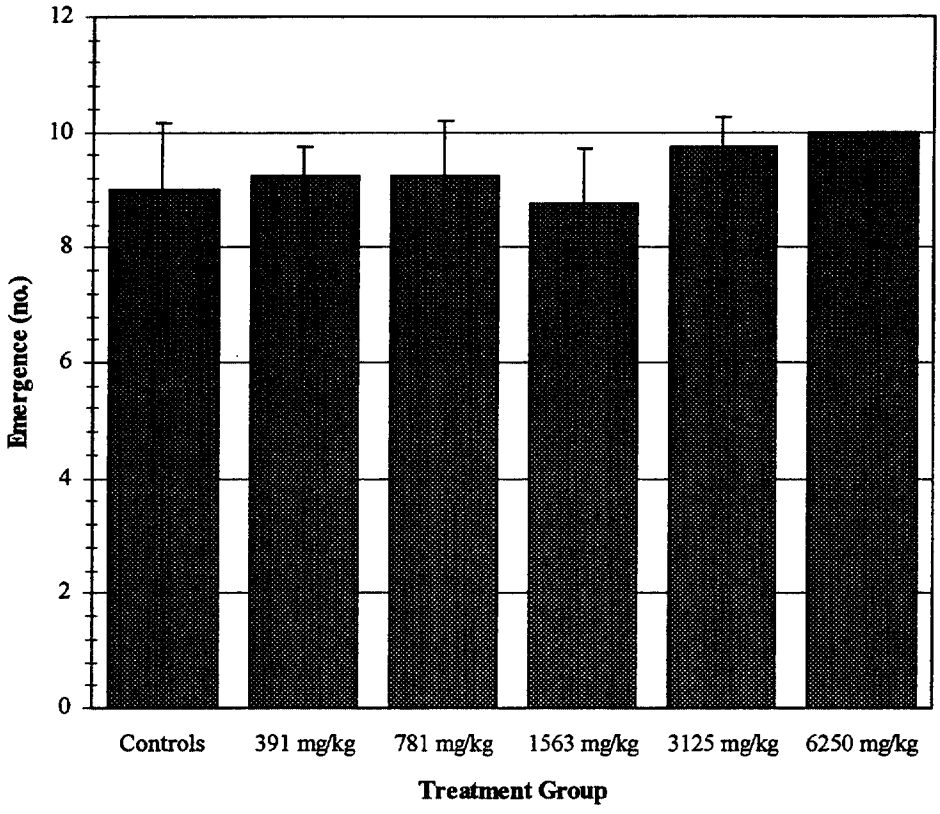
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	10	8	*4*	9.00	1.15
391 mg/kg	9	9	9	10	*4*	9.25	0.50
781 mg/kg	8	9	10	10	*4*	9.25	0.96
1563 mg/kg	9	8	8	10	*4*	8.75	0.96
3125 mg/kg	10	10	10	9	*4*	9.75	0.50
6250 mg/kg	10	9	10	10	*4*	9.75	0.50

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	10	8	*4*	9.00	1.15
391 mg/kg	9	9	9	10	*4*	9.25	0.50
781 mg/kg	8	9	10	10	*4*	9.25	0.96
1563 mg/kg	9	8	8	10	*4*	8.75	0.96
3125 mg/kg	10	10	10	9	*4*	9.75	0.50
6250 mg/kg	10	10	10	10	*4*	10.00	0.00

Appendix 9.2

Mean RYEGRASS Emergence on Day 21



Appendix 9.3

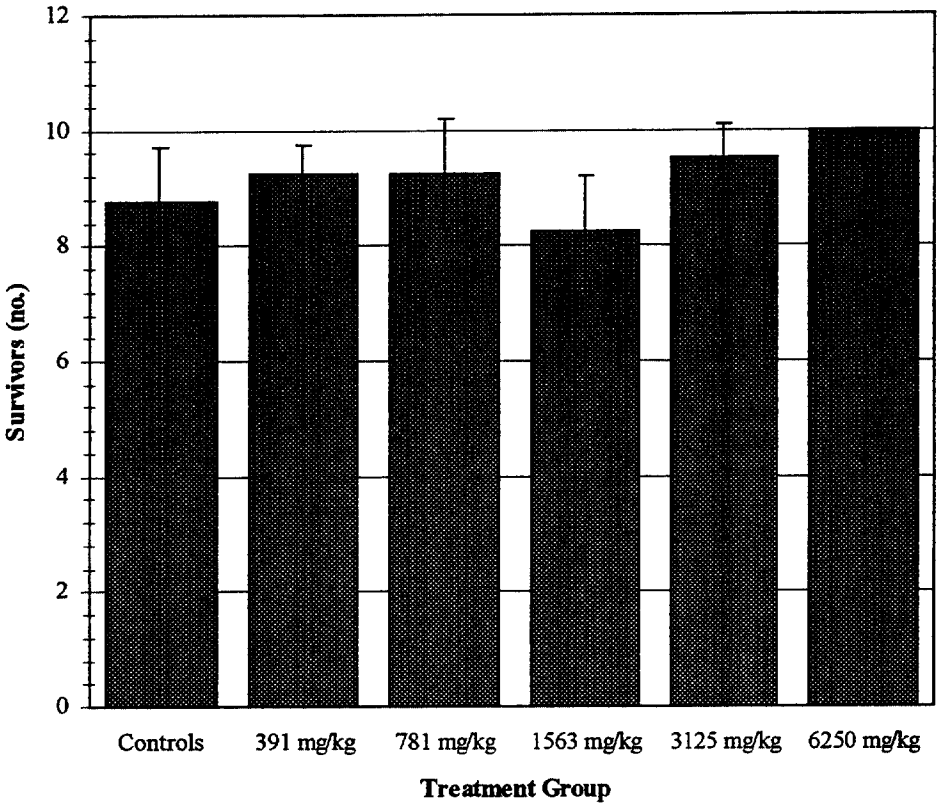
RYEGRASS 21-Day Survival

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	8	10	8	*4*	8.75	0.96
391 mg/kg	9	9	9	10	*4*	9.25	0.50
781 mg/kg	8	9	10	10	*4*	9.25	0.96
1563 mg/kg	9	7	8	9	*4*	8.25	0.96
3125 mg/kg	9	10	10	9	*4*	9.50	0.58
6250 mg/kg	10	10	10	10	*4*	10.00	0.00

Appendix 9.4

Mean RYEGRASS 21-Day Survival



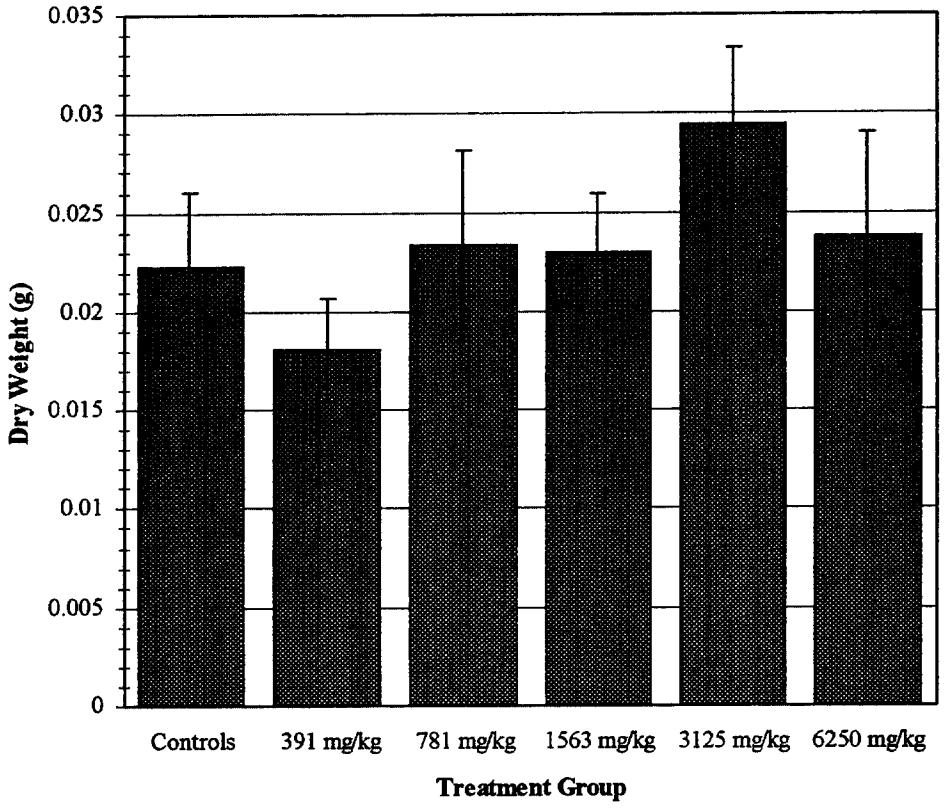
Appendix 9.5

RYEGRASS Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.0213	0.0217	0.0185	0.0276	*4*	0.0222	0.00383
391 mg/kg	0.0211	0.0161	0.0158	0.0194	*4*	0.0181	0.00257
781 mg/kg	0.0196	0.0214	0.0219	0.0304	*4*	0.0233	0.00485
1563 mg/kg	0.0238	0.0186	0.0238	0.0257	*4*	0.0230	0.00303
3125 mg/kg	0.0262	0.0316	0.0337	0.0261	*4*	0.0294	0.00389
6250 mg/kg	0.0254	0.0246	0.0164	0.0289	*4*	0.0238	0.00528

Appendix 9.6

Mean RYEGRASS Dry Weight



Appendix 9.7

RYEGRASS Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	13	15	16	7	12	12	13	11	14	.	*9*	12.6	2.60
	B	.	.	11	13	13	12	12	15	15	11	*8*	12.8	1.58
	C	18	11	17	13	11	14	15	13	16	4	*10*	13.2	3.99
	D	.	.	10	11	10	15	18	15	17	15	*8*	13.9	3.14
391 mg/kg	A	.	10	11	12	14	14	12	12	14	14	*9*	12.6	1.51
	B	.	13	12	13	12	14	6	9	11	10	*9*	11.1	2.47
	C	.	10	12	16	12	13	13	13	11	6	*9*	11.8	2.73
	D	9	16	15	13	14	15	9	13	14	9	*10*	12.7	2.71
781 mg/kg	A	.	.	9	10	12	9	14	13	16	11	*8*	11.8	2.49
	B	.	13	17	14	15	13	10	11	9	15	*9*	13.0	2.60
	C	13	12	14	12	11	16	15	14	14	13	*10*	13.4	1.51
	D	13	19	14	16	10	14	14	16	10	17	*10*	14.3	2.87
1563 mg/kg	A	.	15	5	13	9	14	13	14	11	7	*9*	11.2	3.49
	B	.	.	13	13	14	12	16	13	11	.	*7*	13.1	1.57
	C	.	.	12	14	9	13	6	13	10	14	*8*	11.4	2.83
	D	18	10	9	12	11	15	12	13	13	.	*9*	12.6	2.70
3125 mg/kg	A	17	13	13	13	15	16	12	13	6	.	*9*	13.1	3.14
	B	21	16	16	15	17	15	16	17	13	14	*10*	16.0	2.16
	C	12	20	15	15	14	15	15	17	15	21	*10*	15.9	2.73
	D	.	13	22	16	16	16	13	20	17	15	*9*	16.4	2.96
6250 mg/kg	A	12	1	13	14	14	16	15	13	14	15	*10*	12.7	4.27
	B	13	17	17	14	12	19	14	11	16	9	*10*	14.2	3.08
	C	5	17	15	15	15	10	13	11	12	10	*10*	12.3	3.50
	D	15	18	15	20	20	17	15	15	10	19	*10*	16.4	3.06

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

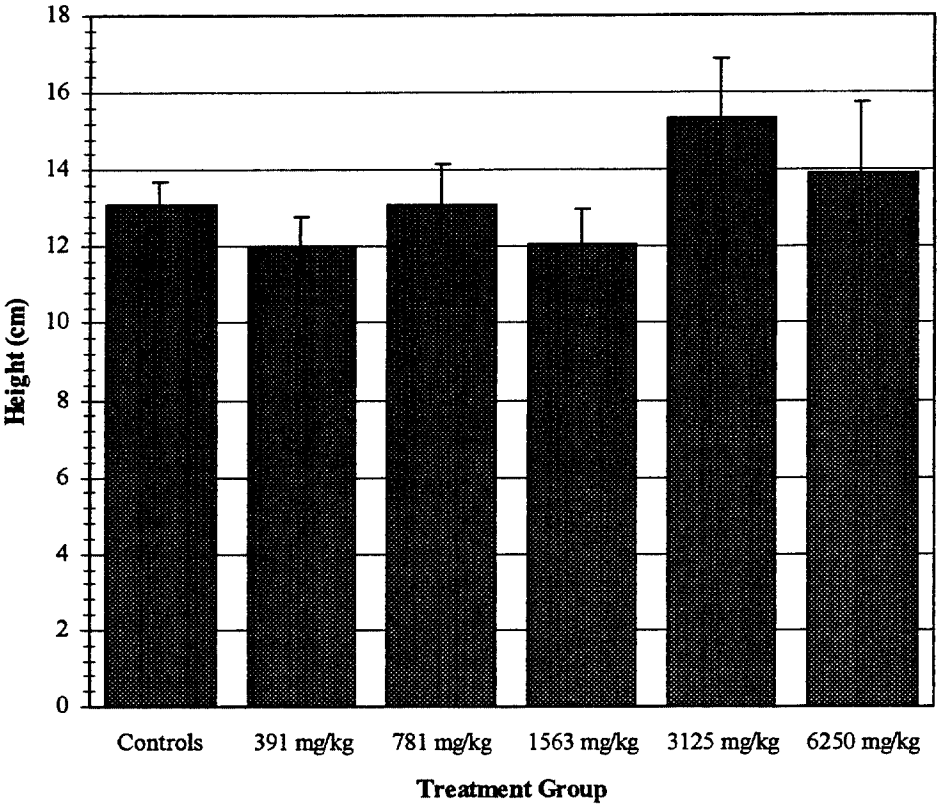
Appendix 9.8

RYEGRASS Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	12.6	12.8	13.2	13.9	*4*	13.1	0.59
391 mg/kg	12.6	11.1	11.8	12.7	*4*	12.0	0.74
781 mg/kg	11.8	13.0	13.4	14.3	*4*	13.1	1.06
1563 mg/kg	11.2	13.1	11.4	12.6	*4*	12.1	0.93
3125 mg/kg	13.1	16.0	15.9	16.4	*4*	15.4	1.52
6250 mg/kg	12.7	14.2	12.3	16.4	*4*	13.9	1.86

Appendix 9.9

Mean RYEGRASS Height on Day 21



Appendix 9.10

RYEGRASS Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	50.N	*10*	5	15.8
	D	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
391 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	40.N	0.-	0.-	0.-	*9*	4	13.3
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
781 mg/kg	A	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
1563 mg/kg	A	.	0.-	40.N	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	4	13.3
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*8*	13	35.4
	C	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
3125 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
6250 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

Appendix 10.1

Soybean Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	10	*4*	10.00	0.00
391 mg/kg	10	10	10	10	*4*	10.00	0.00
781 mg/kg	9	10	10	10	*4*	9.75	0.50
1563 mg/kg	7	10	5	7	*4*	7.25	2.06
3125 mg/kg	9	10	10	10	*4*	9.75	0.50
6250 mg/kg	10	9	10	10	*4*	9.75	0.50

Day 14

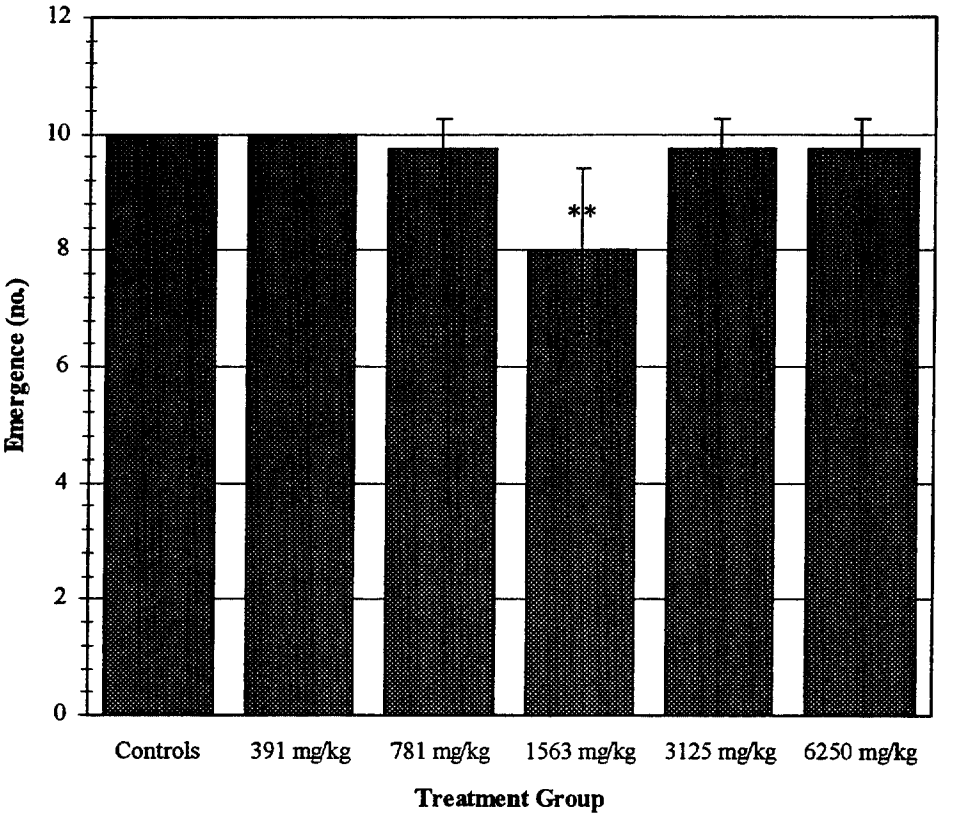
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	10	*4*	10.00	0.00
391 mg/kg	10	10	10	10	*4*	10.00	0.00
781 mg/kg	9	10	10	10	*4*	9.75	0.50
1563 mg/kg	8	10	7	7	*4*	8.00	1.41
3125 mg/kg	9	10	10	10	*4*	9.75	0.50
6250 mg/kg	10	9	10	10	*4*	9.75	0.50

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	10	*4*	10.00	0.00
391 mg/kg	10	10	10	10	*4*	10.00	0.00
781 mg/kg	9	10	10	10	*4*	9.75	0.50
1563 mg/kg	8	10	7	7	*4*	8.00	1.41
3125 mg/kg	9	10	10	10	*4*	9.75	0.50
6250 mg/kg	10	9	10	10	*4*	9.75	0.50

Appendix 10.2

Mean Soybean Emergence on Day 21



** Treatment group mean is significantly different from control mean (Dunnett's test, $p<0.05$)

Appendix 10.3

Soybean 21-Day Survival

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	10	*4*	10.00	0.00
391 mg/kg	9	10	10	10	*4*	9.75	0.50
781 mg/kg	9	10	10	10	*4*	9.75	0.50
1563 mg/kg	8	10	7	7	*4*	8.00	1.41
3125 mg/kg	9	10	10	10	*4*	9.75	0.50
6250 mg/kg	10	9	10	10	*4*	9.75	0.50

Appendix 9.10

Ryegrass Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	30.N	*9*	3	10.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
94 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
188 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
375 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
750 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	40.N	*9*	4	13.3
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
1500 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0

[1]The "ne" indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity
N - Necrosis

Appendix 10

Test Results, SOYBEAN

Appendix 10.1

Soybean Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	7	7	7	6	*4*	6.75	0.500
94 mg/kg	5	8	8	8	*4*	7.25	1.500
188 mg/kg	6	4	9	8	*4*	6.75	2.217
375 mg/kg	7	8	6	8	*4*	7.25	0.957
750 mg/kg	6	7	8	6	*4*	6.75	0.957
1500 mg/kg	6	9	9	8	*4*	8.00	1.414

Day 14

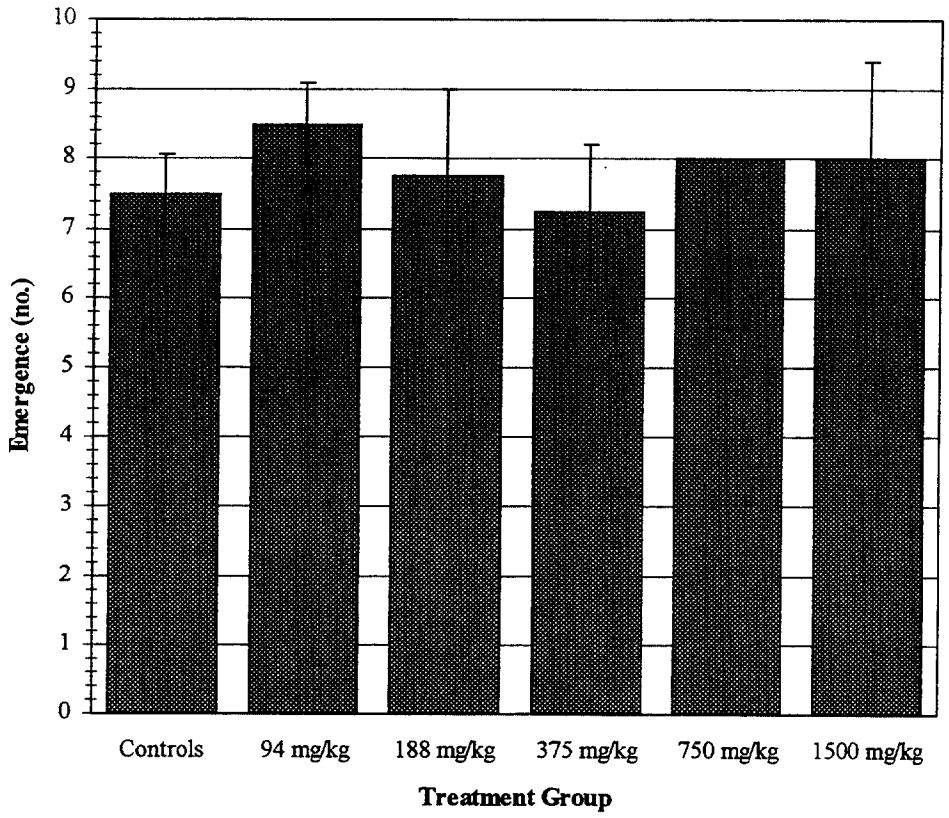
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	7	7	7	7	*4*	7.00	0.000
94 mg/kg	8	8	8	9	*4*	8.25	0.500
188 mg/kg	6	7	9	8	*4*	7.50	1.291
375 mg/kg	7	8	6	8	*4*	7.25	0.957
750 mg/kg	8	8	8	8	*4*	8.00	0.000
1500 mg/kg	6	9	9	8	*4*	8.00	1.414

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	8	7	7	*4*	7.50	0.577
94 mg/kg	8	9	8	9	*4*	8.50	0.577
188 mg/kg	6	8	9	8	*4*	7.75	1.258
375 mg/kg	7	8	6	8	*4*	7.25	0.957
750 mg/kg	8	8	8	8	*4*	8.00	0.000
1500 mg/kg	6	9	9	8	*4*	8.00	1.414

Appendix 10.2

Mean Soybean Emergence on Day 21



Appendix 10.3

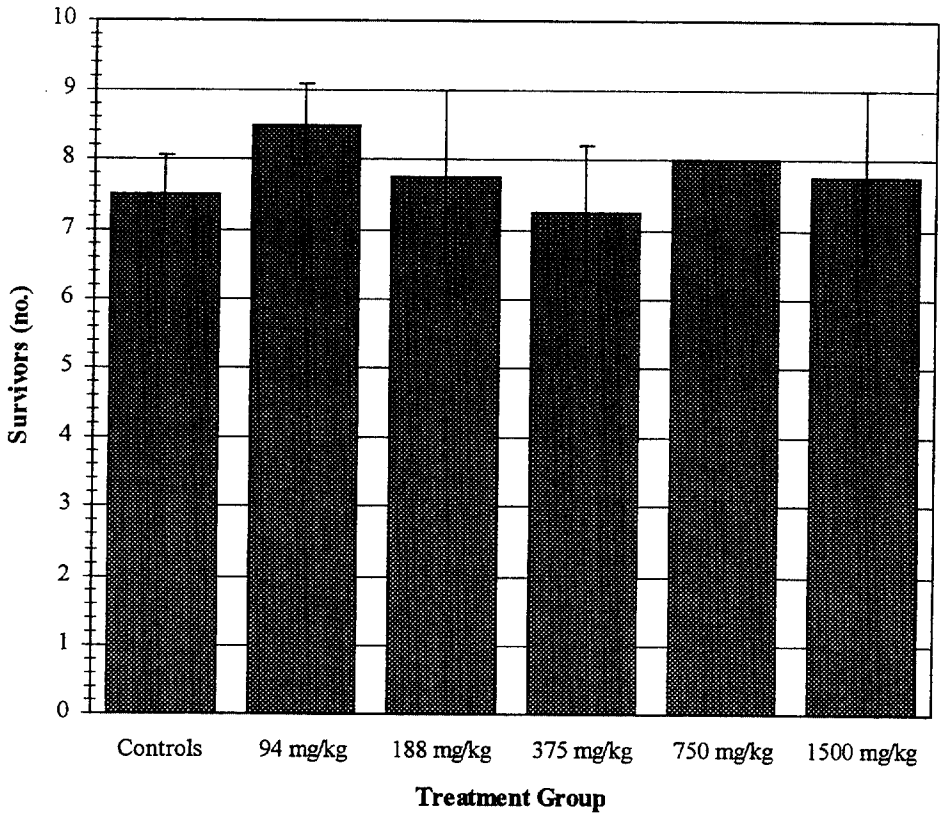
Soybean 21-Day Survival

Day 21

Treatment Group	Number of Surviving Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	8	7	7	*4*	7.50	0.577
94 mg/kg	8	9	8	9	*4*	8.50	0.577
188 mg/kg	6	8	9	8	*4*	7.75	1.258
375 mg/kg	7	8	6	8	*4*	7.25	0.957
750 mg/kg	8	8	8	8	*4*	8.00	0.000
1500 mg/kg	6	9	8	8	*4*	7.75	1.258

Appendix 10.4

Mean Soybean 21-Day Survival



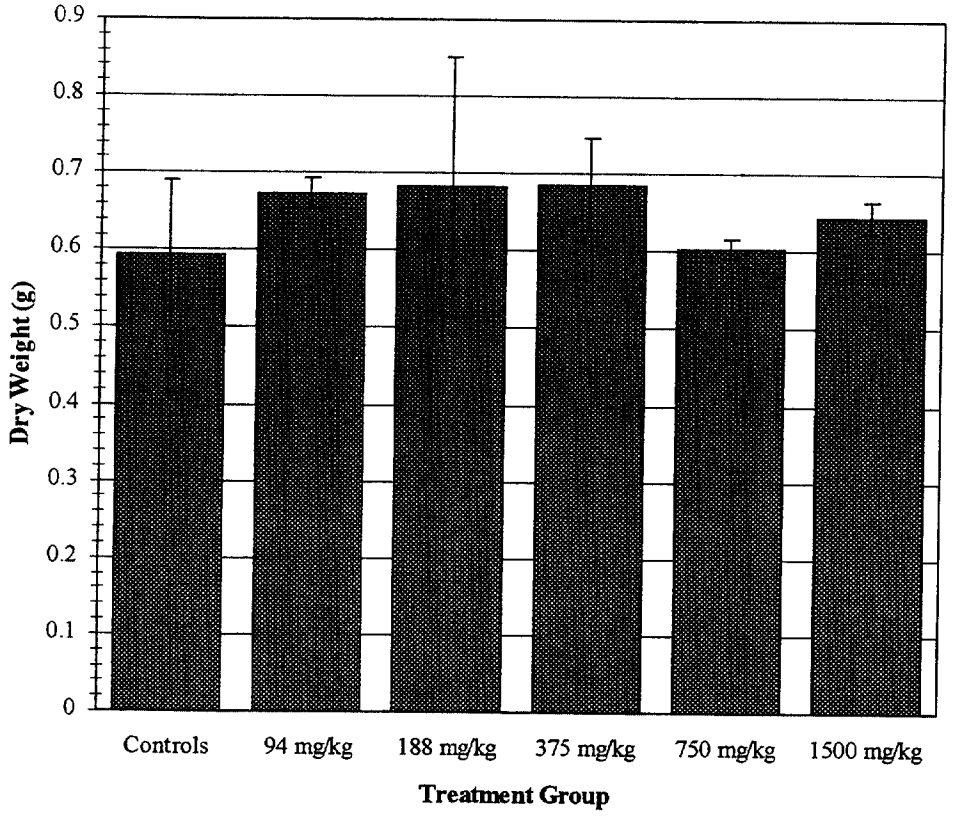
Appendix 10.5

Soybean Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.5848	0.4975	0.7309	0.5545	*4*	0.5919	0.09947
94 mg/kg	0.6713	0.6695	0.6518	0.7009	*4*	0.6734	0.02034
188 mg/kg	0.8676	0.4686	0.7508	0.6425	*4*	0.6824	0.16957
375 mg/kg	0.7036	0.6485	0.7624	0.6280	*4*	0.6856	0.06030
750 mg/kg	0.5994	0.5901	0.6083	0.6200	*4*	0.6044	0.01279
1500 mg/kg	0.6612	0.6143	0.6485	0.6536	*4*	0.6444	0.02073

Appendix 10.6

Mean Soybean Dry Weight



Appendix 10.7

Soybean Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	.	22	25	19	22	22	22	25	21	*8*	22.3	1.98
	B	.	.	14	19	19	21	22	14	23	26	*8*	19.8	4.20
	C	.	.	.	24	24	26	27	22	26	30	*7*	25.6	2.57
	D	.	.	.	26	16	22	26	23	24	26	*7*	23.3	3.59
94 mg/kg	A	.	.	25	29	25	12	23	26	26	26	*8*	24.0	5.13
	B	.	22	25	28	28	21	26	4	28	27	*9*	23.2	7.66
	C	.	.	24	24	5	3	26	22	21	16	*8*	17.6	8.93
	D	.	22	22	24	24	3	25	29	29	27	*9*	22.8	7.87
188 mg/kg	A	.	.	.	.	24	27	25	23	25	18	*6*	23.7	3.08
	B	.	.	31	21	26	27	2	3	20	14	*8*	18.0	10.85
	C	.	23	27	28	28	23	26	27	26	26	*9*	26.0	1.87
	D	.	.	24	24	25	22	21	21	22	24	*8*	22.9	1.55
375 mg/kg	A	.	.	.	24	24	24	23	22	23	22	*7*	23.1	0.90
	B	.	.	25	22	25	29	20	28	27	26	*8*	25.3	3.01
	C	.	.	.	.	25	23	21	23	25	19	*6*	22.7	2.34
	D	.	.	24	25	27	27	6	27	25	37	*8*	24.8	8.60
750 mg/kg	A	.	.	18	24	21	24	25	21	23	20	*8*	22.0	2.39
	B	.	.	29	28	29	23	16	27	24	24	*8*	25.0	4.34
	C	.	.	26	26	19	25	28	25	27	23	*8*	24.9	2.80
	D	.	.	15	26	24	25	26	29	21	19	*8*	23.1	4.52
1500 mg/kg	A	.	.	.	.	23	23	18	22	23	21	*6*	21.7	1.97
	B	.	24	24	26	25	27	25	31	24	12	*9*	24.2	5.09
	C	.	.	24	24	28	28	25	25	23	22	*8*	24.9	2.17
	D	.	.	13	30	22	27	26	27	25	22	*8*	24.0	5.18

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

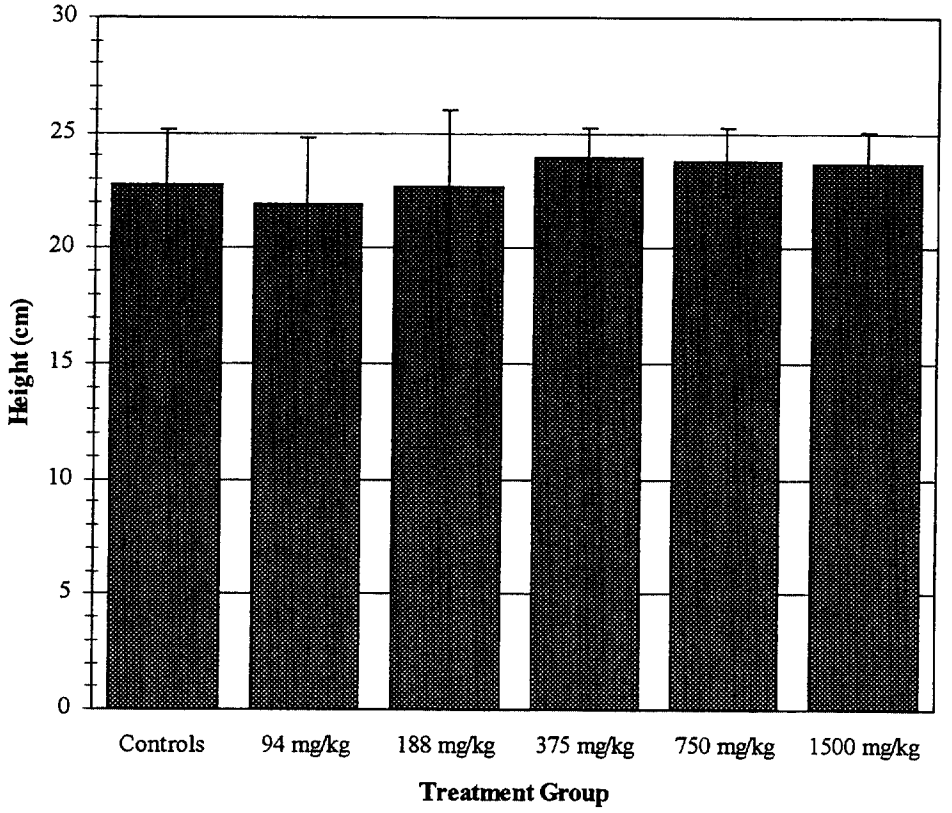
Appendix 10.8

Soybean Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	22.3	19.8	25.6	23.3	*4*	22.7	2.41
94 mg/kg	24.0	23.2	17.6	22.8	*4*	21.9	2.90
188 mg/kg	23.7	18.0	26.0	22.9	*4*	22.6	3.36
375 mg/kg	23.1	25.3	22.7	24.8	*4*	24.0	1.24
750 mg/kg	22.0	25.0	24.9	23.1	*4*	23.8	1.45
1500 mg/kg	21.7	24.2	24.9	24.0	*4*	23.7	1.40

Appendix 10.9

Mean Soybean Height on Day 21



Appendix 10.10

Soybean Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	D	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
94 mg/kg	A	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	D	.	0.-	0.-	0.-	0.-	80.N	0.-	0.-	0.-	0.-	*9*	9	26.7
188 mg/kg	A	.	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	*6*	0	0.0
	B	.	.	0.-	0.-	0.-	0.-	0.-	90.N	0.-	0.-	*8*	11	31.8
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
375 mg/kg	A	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	.	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	*6*	0	0.0
	D	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
750 mg/kg	A	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	D	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
1500 mg/kg	A	.	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	*6*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	.	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	11	33.3
	D	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity

N - Necrosis

Appendix 11

Test Results, TOMATO

Appendix 11.1

Tomato Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	10	7	4	*4*	7.25	2.50
94 mg/kg	5	5	8	8	*4*	6.50	1.73
188 mg/kg	10	9	10	9	*4*	9.50	0.58
375 mg/kg	8	7	9	7	*4*	7.75	0.96
750 mg/kg	8	9	9	8	*4*	8.50	0.58
1500 mg/kg	7	7	10	5	*4*	7.25	2.06

Day 14

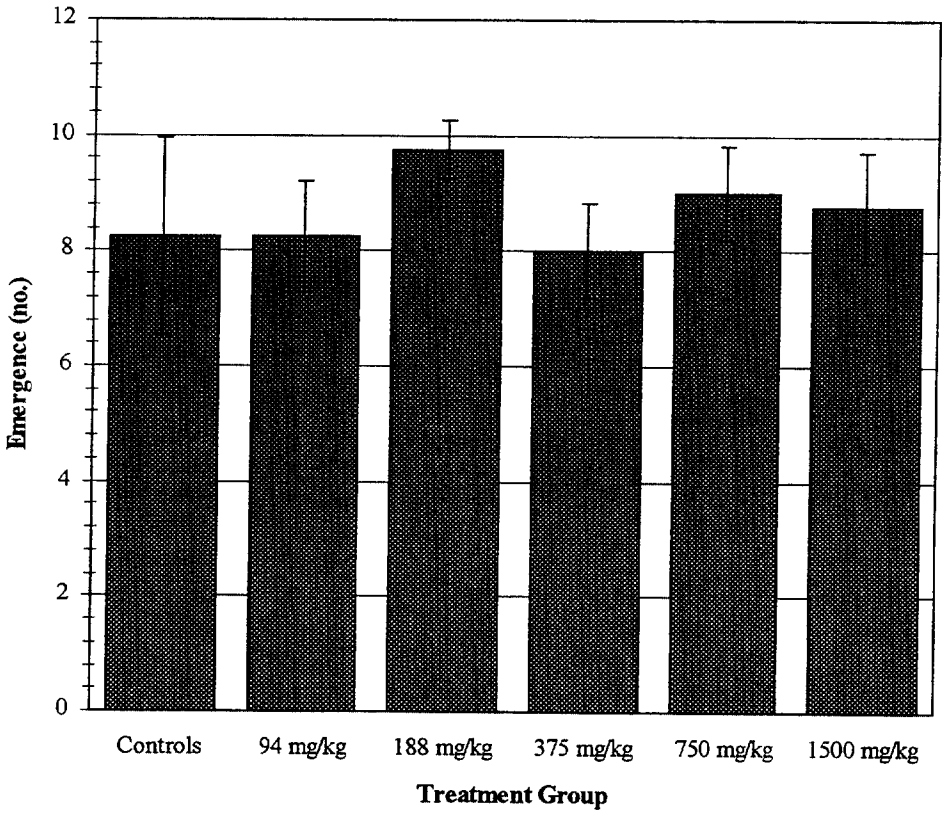
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	8	6	*4*	8.25	1.71
94 mg/kg	7	8	9	8	*4*	8.00	0.82
188 mg/kg	10	10	10	9	*4*	9.75	0.50
375 mg/kg	8	8	9	7	*4*	8.00	0.82
750 mg/kg	8	9	10	9	*4*	9.00	0.82
1500 mg/kg	8	8	10	9	*4*	8.75	0.96

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	8	6	*4*	8.25	1.71
94 mg/kg	7	9	9	8	*4*	8.25	0.96
188 mg/kg	10	10	10	9	*4*	9.75	0.50
375 mg/kg	8	8	9	7	*4*	8.00	0.82
750 mg/kg	8	9	10	9	*4*	9.00	0.82
1500 mg/kg	8	8	10	9	*4*	8.75	0.96

Appendix 11.2

Mean Tomato Emergence on Day 21



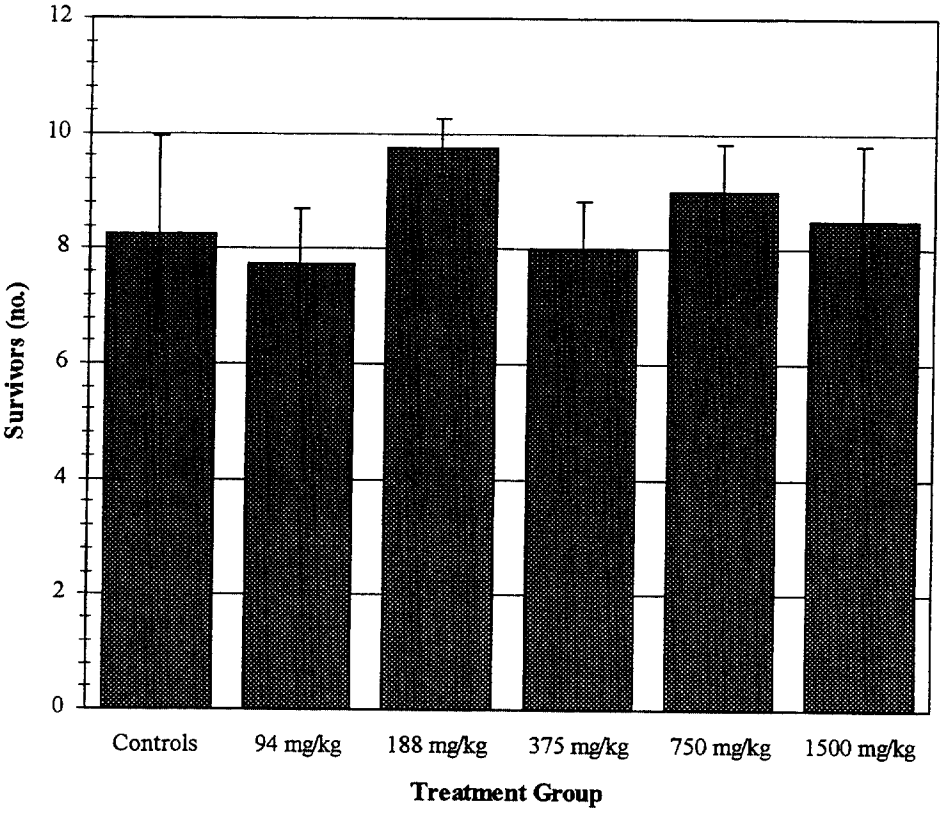
Appendix 11.3

Tomato 21-Day Survival

Treatment Group	Day 21 Number of Surviving Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	8	6	*4*	8.25	1.71
94 mg/kg	7	7	9	8	*4*	7.75	0.96
188 mg/kg	10	10	10	9	*4*	9.75	0.50
375 mg/kg	8	8	9	7	*4*	8.00	0.82
750 mg/kg	8	9	10	9	*4*	9.00	0.82
1500 mg/kg	8	7	10	9	*4*	8.50	1.29

Appendix 11.4

Mean Tomato 21-Day Survival



DECABROMODIPHENYL OXIDE: AN ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439E-106

Organisation for Economic Cooperation and Development
OECD Guideline 209

and

Council of European Communities Directive 67/548/EEC
Annex V, Guideline C.11

AUTHORS:
Edward C. Schaefer
Abul I. Siddiqui

STUDY INITIATION DATE: March 05, 2001

STUDY COMPLETION DATE: August 23, 2001

SUBMITTED TO:

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Decabromodiphenyl Oxide: An Activated Sludge, Respiration Inhibition Test

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439E-106

STUDY COMPLETION: August 23, 2001

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in EPA 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17), and Japan MAFF 59 NohSan, Notification No. 3850, Agricultural Production Bureau, with the following exceptions:

The test substance was not characterized in compliance with Good Laboratory Practice Standards.

The reference substance, obtained from Aldrich Chemical Company (Milwaukee, WI), was not characterized in compliance with Good Laboratory Practice Standards.

The stability of the test and reference substances under conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

The homogeneity and stability of the reference material in the carrier was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

Edward C. Schaefer
Manager, Biodegradation

8/23/2001
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice as published by the U.S. Environmental Protection Agency in EPA 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17); and Japan MAFF 59 NohSan, Notification No, 3850, Agricultural Production Bureau; Wildlife International, Ltd. Standard Operating Procedures and the study protocol. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO: STUDY DIRECTOR:	MANAGEMENT
Test Substance Preparation and D.O. Measurements	March 14, 2001	March 14, 2001	March 19, 2001
Data and Draft Report	March 16, 2001	March 16, 2001	March 30, 2001
Final Report	August 23, 2001	August 23, 2001	August 23, 2001

Robert N. McGee, B.S.
Quality Assurance Representative

August 23, 2001
DATE

REPORT APPROVAL

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Decabromodiphenyl Oxide: An Activated Sludge, Respiration Inhibition Test

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439E-106

STUDY DIRECTOR:

Edward C. Schaefer
Manager, Biodegradation

8/23/2001
DATE

MANAGEMENT:

Henry O. Krueger, Ph.D.
Director, Aquatic Toxicology and Non-Target Plants

8/23/01
DATE

STUDY INFORMATION

Study Initiation Date:	March 05, 2001
Experimental Start Date:	March 14, 2001
Experimental Termination Date:	March 14, 2001
Study Completion Date:	August 23, 2001

Study Director:	Edward C. Schaefer
Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
Sponsor's Representative:	Ms. Wendy Sherman
Study Personnel:	Edward C. Schaefer, B.S., Manager, Biodegradation Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants Abul Siddiqui, B.A., Scientist, Biodegradation

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ABSTRACT

The effect of the test substance on activated sludge microorganisms was assessed by the Activated Sludge Respiration Inhibition Test Method (OECD Guideline 209). The test contained control, reference and treatment groups. The control group was used to determine the background respiration rate of the sludge and was not dosed with the test or reference substance. The reference group was dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at concentrations of 3, 15 and 50 mg/L. The test substance was dosed at a limit concentration of 15 mg/L. After an exposure period of approximately three hours, the respiration rates of the test solutions were measured using a dissolved oxygen meter. The individual respiration rates of the two controls were both 41.6 mg O_2/L/hr. Thus, the difference between the two control respiration rates was 0% and was within the 15% difference limit established for the test. The validity of the test was further supported by the results from the 3,5-dichlorophenol reference group, which resulted in an EC50 of 9.8 mg/L. The EC50 was within the 5 to 30 mg/L range considered acceptable for the test. An average of approximately 1.2 percent inhibition was observed in the treatment group. Following is a summary of the results:

Treatment/Nominal Concentration	Respiration Rate mg O_2/L/hour	Percent Inhibition
Control 1	41.6	NA
Control 2	41.6	NA
3,5-dichlorophenol 3 mg/L	39.0	6.3
3,5-dichlorophenol 15 mg/L	12.8	69.2
3,5-dichlorophenol 50 mg/L	4.9	88.2
Decabromodiphenyl Oxide 15 mg/L	40.0	3.9
Decabromodiphenyl Oxide 15 mg/L	43.4	-4.3
Decabromodiphenyl Oxide 15 mg/L	40.0	3.9

NA – Not applicable

INTRODUCTION

The purpose of this test is to provide a screening method to identify substances that may adversely affect aerobic microbial treatment plants and to indicate suitable non-inhibitory test substance concentrations for use in biodegradability tests.

This study was conducted by Wildlife International, Ltd. for the American Chemistry Council's Brominated Flame Retardant Industry Panel at the Wildlife International, Ltd. biodegradation facility in Easton, Maryland. Original raw data generated by Wildlife International, Ltd. and the original final report are filed under Project Number 439E-106 in the archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to assess the effects of decabromodiphenyl oxide on activated sludge microorganisms by measuring the respiration rate.

EXPERIMENTAL DESIGN

The test contained control, reference, and treatment groups. The control group was used to determine the background respiration rate of the sludge and was not exposed to the test or reference substances. The reference group was dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at concentrations of 3, 15 and 50 mg/L. The test substance was tested at a limit concentration of 15 mg/L, in triplicate.

MATERIALS AND METHODS

This study was conducted according to the procedures outlined in the protocol, "Decabromodiphenyl Oxide: An Activated Sludge, Respiration Inhibition Test," (Appendix II). The protocol was based on the procedures specified in the OECD Guideline for Testing of Chemicals, Method 209 (1) and Council of the European Communities, Guideline C.11, Activated Sludge, Respiration Inhibition Test (2).

Test Substance

The test substance used in this study was a composite of the following three samples:

Manufacturer:	Bromide Compounds Ltd
Sample ID:	Decabromodiphenyl Oxide
Description	White powder
Purity	82% Bromine Content
Batch No.:	980077
CAS No:	1163-19-5
Expiration Date:	Not Given
Date Received:	October 21, 1998
Wildlife International, Ltd. ID:	4667 A & B

Manufacturer:	Great Lakes Chemical Corporation
Sample ID:	Decabromodiphenyl Oxide
Description	White powder
Purity	Not Given
Batch No.:	8480DI30B
CAS No:	1163-19-5
Expiration Date:	Not Given
Date Received:	October 19, 1998
Wildlife International, Ltd. ID:	4664

Manufacturer:	Albemarle Corporation
Sample ID:	SAYTEX 102-E
Description	White powder
Purity	Not Given
Batch No.:	Not Given
CAS No:	1163-19-5
Expiration Date:	Not Given
Date Received:	October 15, 1998
Wildlife International, Ltd. ID:	4663

The composite DBDPO sample was prepared on November 04, 1998 and was assigned Wildlife International Ltd. identification number 4700. The composite sample was prepared by combining equal parts of the three manufacturers' products and mixing for approximately two hours. A sub-sample was shipped to Albemarle Corporation for analysis to determine the characterization and the homogeneity of the mixture.

The test substance was administered to the treatment group by direct weight addition.

Reference Substance

A stock solution of the reference substance, 3,5-dichlorophenol was prepared by dissolving 500 mg in 10 mL of 1N NaOH and then diluting to 30 mL with NANOpure® water. While stirring, enough 1N H_2SO_4 was added to reach the point of incipient precipitation. The solution of 3,5-dichlorophenol then was diluted to 1 L with NANOpure® water. The reference substance was administered by volumetric addition. Following is a description of the reference substance used in this study.

Name:	3,5-dichlorophenol
Manufacturer:	Aldrich Chemical Co., Milwaukee, WI
Lot Number:	02611ES
Physical Description:	White solid
Handling Precautions:	Standard laboratory precautions
Date Received:	January 24, 2000
Expiration Date:	January 24, 2005
Purity:	99.1%
Storage Conditions:	Ambient
CAS Number:	591-35-5
Wildlife International, Ltd. ID:	5179

Test Conditions and Apparatus

Control, reference, and treatment test mixtures were incubated at 20 ± 2°C and aerated for three hours at a rate sufficient to provide aerobic conditions and maintain solids in suspension. The mixtures were prepared and aerated in 500 mL plastic Erlenmeyer flasks and then transferred into 300 mL biochemical oxygen demand (BOD) bottles to conduct the dissolved oxygen (DO) measurements.

Test Inoculum

Activated sludge was collected from the Denton Wastewater Treatment Plant, Denton, Maryland on March 13, 2001. The Denton facility receives wastes from predominately domestic sources. The sludge was sieved using a 2 mm screen and allowed to settle for approximately 30 minutes. After the settling period, the supernatant was removed and the total suspended solids (TSS) concentration of the settled sludge was determined.

The sludge was maintained in the laboratory for 1 day prior to use. Approximately 50 mL of synthetic sewage (Protocol, Appendix II) was added to each liter of activated sludge and the sludge was continuously aerated. Before use, the pH and total suspended solids concentration of the activated sludge were determined.

Procedure

Test mixtures were prepared at 15 minute intervals starting with the first control. The control contained 9.6 mL of synthetic sewage, 120 mL of inoculum, and enough municipal water to bring the total volume up to 300 mL. The mixture was promptly aerated at a rate sufficient to provide aerobic conditions and keep the solids in suspension. Subsequent mixtures contained 9.6 mL of synthetic sewage, 120 mL of inoculum, the appropriate amount of test substance or reference substance stock solution, and enough municipal water to bring the total volume up to 300 mL. Finally, a second control was prepared. All mixtures were aerated for three hours.

Sample Analysis

After three hours of aeration, the contents of the first vessel were transferred to a BOD bottle and the respiration rate was measured over a period of up to 10 minutes. Dissolved oxygen readings were recorded every 10 seconds for 10 minutes or until the DO dropped below 1.0 mg/L, whichever came first using a YSI Model 50B Dissolved Oxygen Meter. The respiration rate in subsequent vessels was determined in an identical manner at 15 minute intervals so that the contact time of the test substance with the activated sludge was three hours.

Calculations

A respiration rate was calculated for each test mixture and expressed in mg O_2/L/hour. The rate was calculated using DO values between approximately 6.5 mg O_2/L and 2.5 mg O_2/L, or over a 10 minute period if the DO did not reach approximately 2.5 mg O_2/L. The respiration rate was calculated using the following equation:

$$\textit{Respiration Rate} = \textit{(initial DO – final DO)/(final time – initial time)}$$

Percent inhibition was calculated using the following equation:

$$\textit{Percent Inhibition} = 1 - \frac{2R_s}{RC_1 + RC_2} \; X\,100$$

where:

R_s = oxygen consumption rate at a given concentration of the test substance
RC_1 = oxygen consumption rate, Control 1
RC_2 = oxygen consumption rate, Control 2

Statistical Analyses

When the dose response pattern allows for the calculation of an EC50 value, the data are analyzed using the computer program of C.E. Stephan (3). The program was designed to calculate the EC50 value and the 95% confidence interval by probit analysis, the moving average, or binomial probability with nonlinear interpolation (4, 5, 6). The EC50 value for the reference group was calculated using nonlinear interpolation.

RESULTS AND DISCUSSION

The temperature range during the maintenance of the sludge and during the test was 20-22°C. The measured total suspended solids (TSS) concentration and pH of the sludge on the day of testing was 4380 mg/L and 7.2, respectively.

Respiration rates and percent inhibitions are presented in Table 1. The respiration rates in the two controls were both 41.6 mg O_2/L/hr. The difference between the two control respiration rates was 0% and was within the 15% difference limit established for the test. The validity of the test was further supported

by the results from the 3,5-dichlorophenol reference group, which resulted in an EC50 of 9.8 mg/L. The EC50 was within the 5 to 30 mg/L range considered acceptable for the test.

Minimal inhibitory effects upon respiration were observed at a decabromodiphenyl oxide concentration of 15 mg/L. The average respiration rate for the treatment group was 41.1 ± 2.0 O_2/L/hr and was slightly lower than that of the control (41.6 ± 0 mg O_2/L/hr). The average percent inhibition observed was approximately 1.2%.

CONCLUSION

Minimal inhibitory effects upon respiration were observed at a decabromodiphenyl oxide concentration of 15 mg/L. The average percent inhibition observed was approximately 1.2%.

REFERENCES

1. **Organisation for Economic Cooperation and Development.** 1989. *Activated Sludge Respiration Inhibition Test*. OECD Guideline 209.

2. **Council of the European Communities.** Directive 67/548/EEC. Annex V. Guideline C.11, *Activated Sludge Respiration Inhibition Test*.

3. **Stephan, C.E.** 1977. "Methods for Calculating an LC50," *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

4. **Finney, D.J.** 1971. *Statistical Methods in Biological Assay*, second edition. Griffin Press, London.

5. **Thompson, W.R.** 1947. *Bacteriological Reviews*, Vol. II, No. 2: 115-145.

6. **Stephan, C.E.** 1977. "Methods for Calculating an LC50," *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

Table 1

Respiration Rates and Percent Inhibitions

Treatment/Nominal Concentration	Respiration Rate mg O_2/L/hour	Percent Inhibition
Control 1	41.6	NA
Control 2	41.6	NA
3,5-dichlorophenol 3 mg/L	39.0	6.3
3,5-dichlorophenol 15 mg/L	12.8	69.2
3,5-dichlorophenol 50 mg/L	4.9	88.2
Decabromodiphenyl Oxide 15 mg/L	40.0	3.9
Decabromodiphenyl Oxide 15 mg/L	43.4	-4.3
Decabromodiphenyl Oxide 15 mg/L	40.0	3.9

NA – Not applicable.

APPENDIX I

Measured Dissolved Oxygen (DO) Concentrations (mg O_2/L)

Time (min./sec.)	Control 1	Reference			Treatment			Control 2
		3 mg/L	15 mg/L	50 mg/L	Rep A 15 mg/L	Rep B 15 mg/L	Rep C 15 mg/L	
00:10	**6.2**	6.7	**8.1**	**8.4**	6.8	**6.5**	6.6	**6.2**
00:20	5.9	**6.4**	8.1	8.5	**6.5**	6.2	**6.5**	5.8
00:30	5.8	6.3	8.1	8.6	6.4	6.1	6.4	5.7
00:40	5.7	6.2	8.1	8.6	6.3	6.0	6.3	5.6
00:50	5.6	6.1	8.0	8.6	6.2	5.9	6.2	5.5
00:60	5.5	6.0	8.0	8.6	6.1	5.8	6.1	5.4
00:70	5.4	5.9	8.0	8.6	6.0	5.6	6.0	5.3
00.80	5.3	5.8	7.9	8.5	5.9	5.5	5.9	5.2
00.90	5.2	5.7	7.9	8.5	5.8	5.4	5.7	5.1
01.00	5.1	5.6	7.9	8.5	5.6	5.3	5.6	5.0
01:10	5.0	5.5	7.9	8.5	5.5	5.2	5.5	4.8
01:20	4.9	5.4	7.8	8.5	5.4	5.1	5.4	4.7
01:30	4.7	5.3	7.8	8.5	5.3	5.0	5.3	4.6
01:40	4.6	5.1	7.7	8.5	5.2	4.9	5.2	4.5
01:50	4.5	5.0	7.7	8.4	5.1	4.7	5.1	4.4
01:60	4.4	4.9	7.7	8.4	5.0	4.6	5.0	4.3
01:70	4.3	4.8	7.6	8.4	4.9	4.5	4.8	4.2
01:80	4.2	4.7	7.6	8.4	4.8	4.4	4.7	4.1
01:90	4.1	4.6	7.6	8.4	4.6	4.3	4.6	4.0
02:00	4.0	4.5	7.5	8.4	4.5	4.2	4.5	3.9
02:10	3.8	4.4	7.5	8.3	4.4	4.0	4.4	3.8
02:20	3.7	4.3	7.4	8.3	4.3	3.9	4.3	3.7
02:30	3.6	4.1	7.4	8.3	4.2	3.8	4.2	3.6
02:40	3.5	4.0	7.4	8.3	4.1	3.7	4.1	3.4
02:50	3.4	3.9	7.3	8.3	4.0	3.6	4.0	3.3
02:60	3.3	3.8	7.3	8.3	3.9	3.5	3.9	3.2
02:70	3.2	3.7	7.3	8.2	3.8	3.4	3.8	3.1
02:80	3.1	3.6	7.2	8.2	3.6	3.2	3.7	3.0
02:90	2.9	3.5	7.2	8.2	3.5	3.1	3.6	2.9
03:00	2.8	3.4	7.1	8.2	3.4	3.0	3.5	2.8
03:10	2.7	3.3	7.1	8.2	3.3	2.9	3.3	2.7
03:20	2.6	3.1	7.1	8.1	3.2	2.8	3.2	2.6
03:30	**2.5**	3.0	7.0	8.1	3.1	2.7	3.1	**2.5**
03:40	2.4	2.9	7.0	8.1	3.0	2.6	3.0	2.4
03:50	2.3	2.8	6.9	8.1	2.9	**2.4**	2.8	2.3
03:60	2.2	2.7	6.9	8.1	2.8	2.3	2.7	2.2
03:70	2.0	2.6	6.9	8.0	2.7	2.2	2.6	2.1
03:80	1.9	**2.5**	6.8	8.0	**2.5**	2.1	**2.5**	2.0
03:90	1.8	2.4	6.8	8.0	2.4	2.0	2.4	1.9
04:00	1.7	2.2	6.8	8.0	2.3	1.9	2.3	1.8
04:10	1.6	2.1	6.7	8.0	2.2	1.8	2.2	1.7
04:20	1.5	2.0	6.7	7.9	2.1	1.7	2.1	1.6
04.30	1.4	1.9	6.6	7.9	2.0	1.6	2.0	1.5
04.40	1.3	1.8	6.6	7.9	1.9	1.5	1.9	1.4
04:50	1.2	1.7	6.6	7.9	1.8	1.4	1.7	1.3
04:60	1.1	1.6	6.5	7.9	1.7	1.3	1.6	1.2
04:70	1.0	1.5	6.5	7.8	1.6	1.2	1.5	1.1
04:80	0.9	1.4	6.4	7.8	1.5	1.1	1.4	1.0
04:90		1.3	6.4	7.8	1.4	1.0	1.3	0.9
05:00		1.2	6.4	7.8	1.3	0.9	1.2	
05:10		1.1	6.3	7.8	1.2		1.1	
05:20		1.0	6.3	7.7	1.1		1.0	
05:30		0.9	6.2	7.7	1.0		0.9	
05:40			6.2	7.7	0.9			
05:50			6.2	7.7				
05:60			6.1	7.7				
05:70			6.1	7.6				
05:80			6.0	7.6				
05:90			6.0	7.6				
06:00			**6.0**	**7.6**				

Bold numbers indicate dissolved oxygen concentrations used to calculate respiration rates.

APPENDIX II

Protocol and Protocol Amendment

PROTOCOL

DECABROMODIPHENYL OXIDE: AN ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST

Organization for Economic Cooperation and Development
OECD Guideline 209

and

Council of European Communities Directive 67/548/EEC
Annex V, Guideline C.11

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

November 28, 2000

Wildlife International, Ltd.

DECABROMODIPHENYL OXIDE: AN ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST

SPONSOR:	American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Edward C. Schaefer
LABORATORY MANAGEMENT:	Henry O. Krueger, Ph.D. Manager of Aquatic Toxicology & Non-Target Plants

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: 3/12/01 Experimental Termination Date: 3/16/01

Project No.: 439E-106

Test Concentrations: 15 mg/L

Test Substance No.: 4700 Reference Substance No. (if applicable): 5179

PROTOCOL APPROVAL

Edward C. Schaefer
STUDY DIRECTOR — DATE 3/05/01

H. Krueger
LABORATORY MANAGEMENT — DATE 3/5/01

Wendy K. Sherman
SPONSOR'S REPRESENTATIVE — DATE 2/19/01

PROTOCOL NO.: 439/112800/ASRIT/SUB439

Wildlife International, Ltd.

INTRODUCTION

The purpose of this test is to provide a screening method to identify substances that may adversely affect aerobic microbial treatment plants and to indicate suitable non-inhibitory test substance concentrations for use in biodegradability tests.

OBJECTIVE

The objective of the study will be to assess the effects of the test substance on activated sludge microorganisms by measuring the respiration rate. An EC50 will be calculated, if possible.

EXPERIMENTAL DESIGN

The test will contain control, reference, and treatment groups. The control group is used to determine the background respiration rate of the sludge and will not be exposed to the test substance. The reference group will be dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at concentrations of 3, 15, and 50 mg/l. The test substance will be tested at a limit concentration of 15 mg/l, in triplicate.

MATERIALS AND METHODS

Test methods are based on the procedures specified in the OECD Guideline for Testing of Chemicals, Method 209 (1) and Council of the European Communities, Guideline C.11, Activated Sludge, Respiration Inhibition Test (2).

Test Substance

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLP), 40 CFR Part 160.31. The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test substance has been characterized according to GLPs prior to using in the test. The attached form **IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR** (Appendix II) is to be used to provide information necessary for GLP compliance. If written verification of GLP test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Wildlife International, Ltd.

The test substance will be administered by direct weight addition. Direct weight addition is the most appropriate route of administration of insoluble materials.

Stock Solution Preparation

A stock solution of 3,5-dichlorophenol will prepared by dissolving 500 mg in 10 mL of 1N NaOH and then diluting to 30 mL with NANO™pure water. While stirring, enough 1N H_2SO_4 (approximately 8 mL) will be added to reach the point of incipient precipitation. The solution of 3,5-dichlorophenol then will be diluted to 1 L with NANO™pure water. The reference substance will be administered by volumetric addition.

Test Conditions and Apparatus

Control, reference, and treatment test mixtures will be incubated at 20 ± 2°C and aerated for 3 hours at a rate sufficient to maintain solids in suspension. The mixtures will be prepared and aerated in 500 mL plastic Erlenmeyer flasks and then transferred into a 300 mL Biochemical Oxygen Demand (BOD) bottle to conduct dissolved oxygen (DO) measurements.

Test Inoculum

Activated sludge from the Denton Wastewater Treatment Plant, Denton, Maryland will be used as the inoculum for the test. The sludge will be sieved using a 2 mm screen and then allowed to settle for approximately 30 minutes. The supernatant above the settled solids will be drained and the total suspended solids (TSS) concentration of the settled sludge will be determined. Based on the result, the concentration of the sludge will be adjusted to 4000 mg/L (± 10%) by diluting with Nanopure® water.

If the sludge cannot be used on the day of collection or if the same batch is required to be used on subsequent days (maximum four days), 50 mL of synthetic sewage (Appendix II) will be added to each liter of activated sludge at the end of each working day. The sludge will be aerated overnight at 20 ± 2°C. Before use, the pH and total suspended solids concentration of the activated sludge will be determined and, if necessary, adjusted to pH 6.0 - 8.0 and a solids concentration of 4000 mg/L (± 10%).

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Procedure

Test mixtures will be prepared at 15 minute intervals starting with the first control. The control will contain 9.6 mL of synthetic sewage, 120 mL of inoculum and enough municipal water to bring the total volume up to 300 mL. The mixture will be promptly aerated at a rate sufficient to keep the solids in suspension. Subsequent mixtures will contain 9.6 mL of synthetic sewage, 120 mL of inoculum, the appropriate amount of test or reference substance, and enough municipal water to bring the total volume up to 300 mL. Finally, a second control will be prepared. All mixtures will be aerated for three hours.

Sample Analysis

After three hours of aeration, the contents of the first vessel will be transferred to a BOD bottle and the respiration rate will be measured over a period of up to 10 minutes. Dissolved oxygen readings will be recorded every 10 seconds for 10 minutes or until the DO drops below 1.0 mg/L, which ever occurs first. The respiration rate in subsequent vessels will be determined in an identical manner at 15 minute intervals so that the contact time of the test substance with the activated sludge is three hours.

Calculations

A respiration rate will be calculated for each test mixture and expressed in mg O_2/L/hour. The rate will be calculated using DO values between approximately 6.5 mg O_2/L and 2.5 mg O_2/L, or over a 10 minute period if the DO does not reach approximately 2.5 mg O_2/L. The respiration rate will be calculated as follows:

$$\textit{Respiration Rate} = \textit{(initial DO} - \textit{final DO)/(final time} - \textit{initial time)}$$

The percent inhibition for each test substance concentration will be calculated using the following equation and plotted against concentration on log paper:

$$\textit{Percent Inhibition} = 1 - \frac{2R_s}{RC_1 + RC_2} \; X \; 100$$

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where

R_s = oxygen consumption rate at a given concentration of the test substance

RC_1 = oxygen consumption rate, Control 1

RC_2 = oxygen consumption rate, Control 2

An EC50 value will be derived, if possible, based on the percent inhibition versus test substance concentration. Confidence limits (95%) for the EC50 will be determined using standard statistical procedures (3).

Quality Control

The test is considered valid only if the following criteria are met:

- the two control respiration rates are within 15% of each other;
- the EC50 (3 hours) of 3,5-dichlorophenol is in the accepted range of 5 to 30 mg/L.

RECORDS TO BE MAINTAINED

Records to be maintained will include, but not limited to, the following:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance as provided by Sponsor.
3. Test initiation and termination dates.
4. Experimental initiation and termination dates.
5. Stock solution concentration calculations and solution preparation.
6. Activated sludge source and pretreatment details.
7. Test temperature and duration.
8. Reference substance results.
9. All dissolved oxygen measurements.
10. Temperature range recorded during test period.
11. Inhibition curve and method for calculation of EC50.
12. If calculated, EC50 and 95% confidence limits.
13. A copy of the final report.

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FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report is to include, but is not limited to, the following when applicable:

1. Name and address of facility performing the study.
2. Dates on which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
5. Identification and characterization of the test substance as provided by Sponsor including name, CAS number, percent active, and other characteristics, if provided by the Sponsor.
6. A description of the transformations and calculations performed on the data.
7. A description of the methods used and reference to any standard method employed.
8. A description of the test system.
9. A description of the preparation of the test solutions, the testing concentration(s), the route of administration, and the duration of the test.
10. A description of all circumstances that may of affected the quality or integrity of the data.
11. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
12. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
13. The location where the raw data and final report are to be stored.
14. A statement prepared by the Quality Assurance Unit listing the dates that the study inspections and audits were made and the dates of any findings were reported to the Study Director and Management.
15. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed by the Study Director.
16. A copy of the signed protocol and amendments.

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CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160); OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

Wildlife International, Ltd.

REFERENCES

1 **Organisation for Economic Cooperation and Development.** 1989. *Activated Sludge Respiration Inhibition Test.* OECD Guideline 209.

2 **Council of the European Communities.** Directive 67/548/EEC. Annex V. Guideline C.11, *Activated Sludge Respiration Inhibition Test.*

3 **Stephan, C.E.** 1977. "Methods for Calculating an LC50," *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

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APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): _____

Test Substance Sample Code or Batch Number: _____

Test Substance Purity (% Active Ingredient): _____ Expiration Date: _____

Solubility: Water: _____ Theoretical Carbon Content : _____

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? Yes____No_____

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd.

_____

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes____No_____
Other pertinent stability information:

_____

IV. Test Concentrations:

Adjust test concentration to 100% a.i.
____ based upon the purity (%) given above.

Do not adjust test concentration to 100%
____ a.i. Test the material AS IS.

V. Toxicity Information:

Mammalian: Rat LD50 _____ Mouse LD50 _____

Aquatic: Invertebrate Toxicity (EC/LC50) _____

Fish Toxicity (LC50) _____

Other Toxicity Information (including findings of chronic and subchronic tests):

_____

VI. Classification of the Compound:

_____ Insecticide _____ Herbicide _____ Fungicide _____
_____ Microbial Agent_____ Economic Poison _____

Other: _____

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APPENDIX II. SYNTHETIC SEWAGE

The synthetic sewage provides the necessary nutrients required for bacterial metabolism. It is prepared by dissolving the following amounts of substances in 1 liter of municipal water:

16.0 g peptone
11.0 g meat extract
3.0 g urea
0.7 g NaCl
0.4 g $CaCl_2$ $2H_2O$
0.2 g $MgSO_4$ $7H_2O$
2.8 g K_2HPO_4

Reagent grade chemicals or better will be used when available. The constituents of the synthetic sewage are not known to contain any contaminants that are reasonable expected to be present and are known to be capable of interfering with the study.

WILDLIFE INTERNATIONAL LTD.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: DECABROMODIPHENYL OXIDE: AN ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST

PROTOCOL NO.: 439/112800/ASRIT/SUB439 **AMENDMENT NO.:** 1

SPONSOR: American Chemistry Council's **PROJECT NO.:** 439E-106

EFFECTIVE DATE: August 16, 2001

AMENDMENT: Calculations, page -6-

DELETE: Confidence limits (95%) for the EC50 will be determined using standard statistical procedures (3).

REASON: The standard statistical procedures identified in the study protocol are not appropriate for calculating 95% confidence limits with the data from the study.

STUDY DIRECTOR — DATE 8/16/2001

LABORATORY MANAGEMENT — DATE 8/16/01

SPONSOR'S REPRESENTATIVE — DATE 8/20/01

Reviewed By QA 8/17/01

APPENDIX III

Test Substance Characterization

ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT

FINAL REPORT ON THE CHEMICAL CHARACTERIZATION OF DECABROMODIPHENYL OXIDE (DBDPO) IN SUPPORT OF A STUDY OF "DECABROMODIPHENYL OXIDE: AN ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST"

I.	Reference Protocol Number:	DBDPORESPIR-01-26-2001
II.	Sponsor:	American Chemistry Council Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209 Study Monitor: Wendy K. Sherman
III.	Analytical Testing Facilities:	Albemarle Corporation Albemarle Technical Center 8000 GSRI Avenue Baton Rouge, LA 70820 Study Chemist: Paul F. Ranken, Ph. D.
IV.	Dates of Performance:	Study initiation date: January 26, 2001 Interim report issued: March 13, 2001 Final report issued: August 8, 2001
V.	Test Article:	Decabromodiphenyl oxide (WIL Test Substance 4700). The test article is a composite of commercial product from Albemarle Corporation, Great Lakes Chemical Corporation and Ameribrom (the Dead Sea Bromine Group). The composite was prepared by Wildlife International Ltd., Easton, MD 21601.
VI.	Objective/Methodology:	This study was initiated to confirm the identity of the test article, to determine the purity of the test article and to confirm the stability of the

test article during the study of "Decabromodiphenyl Oxide: An Activated Sludge, Respiration Inhibition Test." The identity of the test article sample was confirmed by Fourier Transform Infrared Spectroscopy using SOP No. ARS 284-R4. In this procedure, the test article sample infrared spectrum was compared to a standard reference spectrum of decabromodiphenyl oxide. The reference infrared spectrum was located in the Aldrich Condensed Phase High Resolution data library. The data library is an electronic collection of infrared spectra given in the Aldrich Library of FT-IR Spectra monographs. The purity (area % decabromodiphenyl oxide) of the test article sample was determined by gas chromatography using SOP No. ARS 325-R1. In this procedure an aliquot of a solution containing the test article sample was injected into a gas chromatograph and the purity of the test article sample was expressed as a percentage (area %). The test article sample was further characterized by using the procedure in SOP No. ARS 325-R1 to measure the concentration (area %) of other brominated impurities. The stability of the test article was determined by comparing the decabromodiphenyl oxide purity (area %) of the pre-study sample with the decabromodiphenyl oxide purity of an end-of-study sample. The stability of the test article was confirmed since the decabromodiphenyl oxide purity (area %) of the pre-study sample and the end-of-study samples did differ by more than 5 %. Chain of Custody and Sample Handling were conducted according to established standard operating procedures.

VII. Protocol Deviations:

One protocol deviation occurred during this study. The protocol required the stability of the test article be demonstrated by comparing the results of the analysis of a study day-zero sample with the results of the analysis of the end-of-study sample. A day zero sample was

not received from the test laboratory. Therefore, the end-of-study sample was analyzed and the results were compared to the results from the analysis of the pre-study sample. This deviation did not affect the quality or the integrity of the data.

VIII. Results:

The attached Conclusions and Test Article Analytical Data contains all of the test results on the test article. The identity of the test article was confirmed by Fourier Transform Infrared Spectroscopy. The purity of the test article was determined to be 97.90 area%. The test article contained three measurable impurities in concentrations of 0.02, 0.24 and 1.84 area %. The stability of the test article was confirmed by GC analyses; the decabromodiphenyl oxide concentration (area%) of the pre-study sample differed by less than 5% from the concentration of the end-of-study sample. There were no circumstances that may have affected the quality or integrity of the data.

IX. Regulatory Requirements:

The study conformed to the requirements of EPA TSCA (40 CFR Part 792) Good Laboratory Practice Regulations and the OECD [C(97)186/Final] Good Laboratory Practice Regulations.

X. Data/Record Retention:

All original raw data records will be forwarded to the QAU Coordinator and filed in the designated Health and Environment archives at Albemarle Corporation, Health and Environment Department, 451 Florida Street, Baton Rouge, LA 70801.

XI. Protocol Signatures:

Paul F. Ranken — August 3, 2001

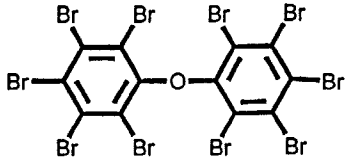
Paul F. Ranken, Ph. D. — DATE

STUDY CHEMIST

TABLE I

CONCLUSIONS AND TEST ARTICLE ANALYTICAL DATA
(Wildlife International Number 4700; WIL #4700)

Chemical Name: Decabromodiphenyl Oxide
CAS Number: 1163-19-5
Molecular Weight: 959.05
Physical Form: White Powder
Chemical Structure:



ANALYSIS	RESULTS			ANALYSIS DATE	ANALYST
FT-IR	The FT-IR spectrum was obtained and it was consistent with the Aldrich standard reference spectrum of pentabromophenyl ether (decabromodiphenyl oxide). All spectra are on file with the original data.			02/14/01	W. T. Cobb
	Pre-Study	End-of-Study	Difference (%)	02/14/01 & 07/23/01	P. E. Smith
Decabromodiphenyl oxide	97.9	97.9	0		

Conclusion: Based on these analytical data, the test article was identified as Decabromodiphenyl Oxide. The test article was 97.9% purity and contained three measurable impurities. The test article was stable during the study of "Decabromodiphenyl Oxide: An Activated Sludge, Respiration Inhibition Test".

Conclusions and Test Article Data. 2.

Characterization of Test Article by GC (Area %)

Pre-Study Sample

	Area %
Decabromodiphenyl Oxide	97.90
Other Brominated Diphenyl Oxide	1.84
Other Brominated Diphenyl Oxide	0.24
Other Brominated Diphenyl Oxide	0.02

End-of-Study Sample

Decabromodiphenyl Oxide	97.93
Other Brominated Diphenyl Oxide	1.78
Other Brominated Diphenyl Oxide	0.28

Attachments

Infrared Spectrum- Test Article Sample
Infrared Spectrum- Decabromodiphenyl Oxide Reference Spectrum
Chromatogram- Pre-Study Test Article Sample
Chromatogram- End-of-Study Test Article Sample